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**New and Revised Chemical
Fate Test Guidelines**

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CHEMICAL FATE TESTING GUIDELINES
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PARTITION COEFFICIENT (n-OCTANOL/WATER)

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDE AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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PARTITION COEFFICIENT (n-OCTANOL/WATER)I. INTRODUCTIONA. Background and Purpose

Bioconcentration, the accumulation of a substance in living tissues or other organic matter as a result of net chemical uptake from the medium (e.g., water), is a factor in determining the movement of a chemical in the environment and the potential effects of the chemical on biota. Hydrophobic chemicals that are present in the aqueous environment at subtoxic concentrations may accumulate to toxic levels once inside organisms, presumably through diffusion into nonpolar cell components, where they accumulate because of their greater solubility. Further movement of the substance in living tissues may occur as a result of ingestion of lower trophic level organisms, i.e., food chain effects.

The tendency of an organic chemical to bioconcentrate in living cells can be inferred from the value of the octanol/water partition coefficient, K_{OW} (Neely et al. 1974). Chemicals with K_{OW} less than 10 will not significantly partition into, or tend to accumulate in, living cells. Chemicals with K_{OW} greater than 10^6 will tend to accumulate. Chemicals that exist in the environment at subtoxic levels may bioconcentrate to toxic levels once inside organisms.

This test guideline describes a detailed and commonly used procedure for determining the octanol/water partition coefficient of organic chemicals.

B. Definitions and Units

The octanol/water partition coefficient (K_{OW}) is defined as the equilibrium ratio of the molar concentrations of a chemical in n-octanol and water, in dilute solution. K_{OW} is a constant for a given chemical at a given temperature. Since K_{OW} is the ratio of two molar concentrations, it is a dimensionless quantity. Sometimes K_{OW} is reported as $\log_{10} K_{OW}$.

The mathematical statement of K_{OW} is:

$$K_{OW} = \frac{C_{\text{octanol}}}{C_{\text{water}}}, \quad (1)$$

where C is the molar concentration of the solute in n-octanol and water at equilibrium at a given temperature.

According to Nernst (1891) the distribution law applies only to individual molecular species in solution. If a molecule dissociates or associates in octanol and water, then equation (1) must be modified. In general, if α represents the fraction of the total solute that is dissociated or associated, assuming that either association or dissociation occurs in each solvent, then

$$K_{OW} = \frac{(1 - \alpha_{\text{oct.}}) C_{\text{oct.}}}{(1 - \alpha_{\text{water}}) C_{\text{water}}}, \quad (2)$$

since $(1 - \alpha)$ gives the fraction of unchanged molecules in each phase. For the special case where no association takes place in octanol, equation (2) reduces to

$$K_{ow} = \frac{C_{oct.}}{(1 - \alpha_{water}) C_{water}}, \quad (3)$$

where α_{water} represents the fraction of the total solute that has dissociated in water.

C. Principle of the Test Method

The conventional method for determining the octanol/water partition coefficient is carried out by distributing a chemical between n-octanol and water in a vessel at constant temperature and measuring the concentration in the two liquid phases after equilibration. Numerous researchers use the conventional method for determining K_{ow} and have published papers using this method (e.g., Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971; Chiou et al. 1977).

D. Applicability and Specificity

The test guideline is designed to determine the octanol/water partition coefficient of solid or liquid organic chemicals in the range 10 to 10^6 . For chemicals whose values lie outside this range, K_{ow} should be characterized as less than 10 or greater than 10^6 with no further quantification.

II. TEST PROCEDURES

A. Test Conditions

1. Special Laboratory Equipment

- (1) A thermostatic bath, chamber, or room with a shaker and temperature control as specified in Temperature Control below;
- (2) an ultracentrifuge with temperature control as specified in Temperature Control below;
- (3) stainless steel or glass centrifuge tubes with sealable caps. Special glass centrifuge tubes can be used up to approximately 12,000 G and stainless steel tubes can be used at high G values;
- (4) a mechanical shaker; and
- (5) a pH meter capable of resolving differences of 0.1 pH unit or less.

2. Temperature Control

It is recommended that the temperature of the water bath, or chamber, or room, and the ultracentrifuge be controlled to $(25 \pm 1)^{\circ}\text{C}$.

3. Solvents

It is extremely important that n-octanol, purified as described in Section II.B.1.a, and distilled or reagent grade water, i.e., ASTM Type II water or an equivalent grade, be used. ASTM Type II water is described in ASTM D-1193-77, "Standard Specification for Reagent Water."

4. Concentration of Solute

It is extremely important that all experiments be carried out at solute concentration $C < 0.01M$ (Molar) in octanol and water and well below the solubility in either phase.

5. Equilibration Time

In general, 1 hour of gentle agitation is sufficient to reach equilibrium. For surfactants, at least 16 hours is required to reach equilibrium.

6. Octanol/Water Volume Ratio

It is recommended that the ratio of the volumes of the two liquids be adjusted as appropriate for the relative solubility of the chemical in octanol and water. By adjusting the volumes, concentration errors (resulting from analytical errors) are minimized and errors resulting from dividing large numbers by small numbers are kept to a minimum.

7. Chemical Analysis of the Octanol and Water Phases

In determining the K_{ow} value for any given solute, it is important that both the octanol and water phases be analyzed for the chemical. An analytical method should be selected that is most applicable to the analysis of the specific chemical. Chromatographic methods are preferable because of their compound specificity in analyzing the parent chemical without interference from impurities. Whenever practicable, the chosen analytical method should have a precision with ± 5 percent.

8. Emulsification and Ultracentrifugation

It is important that gentle shaking be used to minimize the formation of emulsions. Ultracentrifugation is necessary to separate troublesome emulsions and to separate the octanol and water phases. Therefore, it is very important that ultracentrifugation be carried out at 25°C for 20 minutes in a temperature controlled ultracentrifuge. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases can be determined by trial-and-error experimentation.

9. Equilibration Vessel

If feasible, equilibration should be carried out in a centrifuge tube (stainless steel or glass) with a sealable cap. It is important that the centrifuge tubes be almost completely full. In this way, partitioning with air will be minimized,

especially for volatile chemicals, and the mixture will be completely mixed.

Very hydrophobic chemicals, with K_{OW} in the order of 10^4 to 10^6 , require relatively large volumes of the aqueous phase. Hence, for these chemicals, it is recommended that equilibration be carried out in a large ground-glass stoppered flask.

10. Speciation Effects

The octanol/water partition coefficient, K_{OW} , has been defined in Section I.B. The mathematical statement of K_{OW} is given by equation (1).

If the chemical does not associate or dissociate in octanol and water, then use equation (1) and determine K_{OW} at molar concentrations $C < 0.01M$ and $C_1 = 0.01C$.

If the chemical associates in octanol or water or in both liquids, then use equation (1) and determine K_{OW} at molar concentrations $C < 0.01M$, $C_1 = 0.1C$, $C_2 = 0.01C$, $C_3 = 0.001C$ When K_{OW} is constant at two molar concentrations differing by a factor of 10, then the effect of association has been minimized or eliminated.

If a molecule dissociates or associates in octanol and water, then it is extremely important that equation (1) be modified to take into account such speciation changes as ionization, aggregation, and hydration. For the special case, where no association takes place in octanol and only dissociation takes

place in water, equation (3) can be used. For chemicals that reversibly ionize or pronate (e.g., carboxylic acids, phenols, or anilines), use equation (3) with water buffered at pH 5.0, 7.0, and 9.0. It is recommended that buffers described in Section II.B.1.b be used.

11. Prerinsing of all Transfer Vessels

It is important that all transfer vessels be prerinsed with a portion of the equilibrium phase prior to transfer for analysis. This is especially important for very hydrophobic chemicals.

B. Preparations

1. Reagents and Solutions

a. Octanol and Water

Very pure n-octanol can be obtained as follows: wash pure n-octanol (minimum 98 percent pure) sequentially with 0.1N H_2SO_4 , with 0.1N NaOH, then with distilled water until neutral. Dry the n-octanol with magnesium sulfate and distill twice in a good distillation column under reduced pressure [b.p. about 80°C at 0.27 kPa (2 torr)]. It is important that the octanol produced be at least 99.9 percent pure. Alternatively, a grade equivalent to Fisher Scientific Co. No. A-402 "Certified Octanol-1" can be used. It is important that distilled or reagent grade (ASTM Type II) water be used.

b. Buffer Solutions

Prepare buffer solutions using reagent grade chemicals in distilled or reagent grade water as follows:

pH 5.0 - To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust final volume to 500 mL with reagent grade water.

pH 7.0 - To 250 mL of 0.1 potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust final volume to 500 mL with reagent grade water.

pH 9.0 - To 250 mL of 0.07M borax add 69 mL of 0.1M HCl; adjust final volume to 500 mL with reagent grade water.

Check the pH of each buffer solution at 25°C with a pH meter and adjust to pH 5.0, 7.0, or 9.0, if necessary.

c. Presaturation of the Solvents

Before a partitioning experiment is carried out, prepare octanol saturated with water and water saturated with octanol. Add purified n-octanol to a large stock bottle and sufficient distilled water to saturate it. Shake the flask for 24 hours on a mechanical shaker. Then allow sufficient time for the mixture to stand so that the two phases separate. Repeat this procedure

using another large stock bottle containing distilled water and sufficient octanol to saturate it. The desired quantities of the presaturated solvents can be taken from these stock bottles for each partition experiment.

d. Preparation of Test Solution

Prepare a 10^{-2} to 10^{-3} M solution of the test material in octanol.

C. Performance of the Test

- (1) Add a small volume of the octanol test solution (1 to 5 mL) to a centrifuge tube with a sealable cap as described in Section II.A.9.
- (2) Add the required volume of water to the centrifuge tube as described in Section II.A.6. The volume of water required is variable, depending upon the amount of chemical required for the analysis. Generally, 20-40 mL of water should be sufficient. Make sure that the centrifuge tube is almost completely full. In this way, partitioning with air will be minimized. This is important, especially when determining K_{ow} for volatile chemicals.
- (3) Equilibrate the samples at 25°C in a constant temperature bath, chamber, or room by gently shaking the centrifuge tube for 1 hour. Avoid vigorous shaking that may cause troublesome emulsions to form. For surfactants, a

minimum of 16 hours of shaking is required as described in Section II.A.5.

- (4) Centrifuge the samples at 25°C for 20 minutes to break any emulsion and to separate the octanol and water phases. Evidence for breaking the emulsion and separation of the water and octanol phases can be obtained using a turbidimeter. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases can be determined by trial-and-error experimentation.
- (5) Sample the octanol and water phases as follows:
 - o Withdraw by pipet a known volume of the octanol phase (approximately 1/2 or less of the total octanol phase) and transfer to an analysis cell or diluting solvent. Before transferring the aliquot of the octanol phase, wipe the outside of the pipet with a paper tissue.
 - o Remove by pipet the remainder of the octanol phase including the interfacial layer and discard.
 - o Insert another clean pipet close to the bottom of the centrifuge tube and carefully withdraw a known volume of the aqueous phase. Wipe the bottom exterior part of the pipet with a tissue and discharge the aqueous sample directly into an analysis cell or extraction solvent. Do not allow the extraction solvent to contact the pipet stem.

- (6) Select an analytical method that is most applicable to the analysis of the specific chemical as described in Section II.A.7. Determine the concentration in the octanol and water phases. Express the concentration of the chemical in octanol and water in moles/liter (M).
- (7) Determine the partition coefficient in triplicate (steps 1 through 7) at two concentrations of the test material $C < 0.01M$ and $C_1 = 0.1C$ as described in Section II.A.10. If K_{ow} is not constant at C and C_1 , then association effects should be considered. Therefore, follow steps 1 through 7 at lower concentrations until K_{ow} is constant at two concentrations differing by a factor of 10 as described in Section II.A.10.
- (8) Very hydrophobic chemicals (with K_{ow} on the order of 10^4 to 10^6) required relatively large volumes of the aqueous phase as described in Section II.A.5 and Section II.A.9. Hence, for very hydrophobic materials, equilibrate the octanol and water phases in a large ground-glass stoppered flask as described above in step (3). For the final phase separation, transfer the two phase mixture to centrifuge tubes that have been prerinsed with some of the aqueous phase; centrifuge as described in step (4); withdraw aliquots from each centrifuge tube as described in step (5); and recombine for analysis. [Note: Prerinse all transfer tubes with the water phase.] Complete steps (6) and (7) to determine K_{ow} .

- (9) For materials that reversibly ionize or protanate, determine K_{ow} at pH 5.0, 7.0, and 9.0 as described in Section II.A.10. Follow steps (1) through (7) using the buffered aqueous solutions described in Section II.B.1.b. Using the acid dissociation constant and the concentration of the chemical in the aqueous phase $[C_{water}]$, the term a can be calculated. The concentration of undissociated chemical can be determined from α and C_{water} .

III. DATA AND REPORTING

A. Test Report

For each individual determination, report the octanol/water partition coefficient at each concentration of the test substance, including the molar concentration of chemical in each phase [C_{oct} and C_{water}]. In addition, report the mean value of K_{ow} , and the standard deviation.

B. Specific Analytical and Recovery Procedures

- (1) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision; and
- (2) if extraction methods were used to separate the solute from the octanol and aqueous phases, provide a description of the extraction data.

C. Other Test Conditions

Report the experimental (G) value required to break the emulsion and to achieve separation of the octanol and water phases.

IV. REFERENCES

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PARTITION COEFFICIENT (n-OCTANOL/WATER)

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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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I. NEED FOR THE TEST

Bioconcentration, the accumulation of a substance in living tissues or other organic matter as a result of net chemical uptake from the medium (e.g., water), is a factor in determining the movement of a chemical in the environment and the potential effects of the chemical on biota. Hydrophobic chemicals that are present in the aqueous environment at subtoxic concentrations may accumulate to toxic levels once inside organisms, presumably through diffusion into nonpolar cell components, where they accumulate because of their greater solubility. Further movement of the substance in living tissues may occur as a result of ingestion of low trophic level organisms, i.e., food chain effects.

The octanol/water partition coefficient K_{OW} has been shown to be a good predictor of the tendency of chemicals to bioconcentrate in fish (Neely et al. 1974). Since 1974, K_{OW} has been used as a measure of bioconcentration potential in fatty tissues in aquatic and other living organisms. The numerical value of the octanol/water partition coefficient is one factor to be considered in determining whether to conduct fish bioconcentration studies. Other factors must also be taken into account. For example, transformation rates (e.g., rates of biodegradation, hydrolysis, photolysis, and oxidation) must also be considered. If a chemical transforms readily by one of these processes, the potential for bioconcentration will be reduced significantly and fish bioconcentration studies may not be needed.

The octanol/water partition coefficient has been introduced by Hansch to correlate biological activity and chemical structure (Hansch 1969; Hansch and Fujita 1964). Numerous papers have been published by Hansch and his coworkers on this subject in the ensuing years. A monograph has been published on the Hansch approach (Gould 1972).

A recent publication has indicated that the sorption of several hydrophobic pollutants on natural sediments can be related to the octanol/water partition coefficient. Karickhoff et al. (1979) showed that a reasonable estimate (within a factor of two) of the sorption behavior of hydrophobic pollutants can be made from knowledge of the particle size distribution and associated organic content of the sediment and the octanol/water partition coefficient.

Another recent publication has described a novel method for estimating the distribution of a chemical in the environment (Mackay 1979). K_{ow} is used in this partitioning analysis. This partitioning analysis will be used as a guide to ecological and health effects testing.

II. SCIENTIFIC ASPECTS

A. Rationale for the Use of the Octanol/Water Partition Coefficient to Estimate Bioconcentration Potential

Intuitively, the absorption and fat storage of xenobiotic chemicals in living organisms seem to be related to lipophilicity or preferential solubility in fats as compared to water. By definition, the octanol/water partition coefficient K_{ow} expresses the equilibrium concentration ratio of an organic chemical partitioned between octanol and water in dilute solution. If one assumes that octanol simulates fats in its solubilizing effect on organic chemicals, then K_{ow} should be a potential measure of the ease of storage of organic chemicals in fats. For example, a large value of K_{ow} indicates that an organic chemical is not very soluble in water but soluble in octanol. Hence, this would suggest the potential for a large storage of the organic chemical in fats. Davies et al. (1975) reported human pesticide poisoning by a fat-soluble organophosphate, dichlofenthion. The octanol/water partition coefficient K_{ow} was found to be very high (1.37×10^5), which correlated with the high fat storage of this chemical.

Neely et al. (1974) found a pronounced correlation between K_{ow} and the bioconcentration in trout muscle. Specifically, these researchers obtained a linear correlation between the log of bioconcentration and the log of calculated K_{ow} for a series of organic chemicals. Since that time, K_{ow} has been used by researchers as an index of bioconcentration potential in living

organisms. The Office of Pesticide Programs (OPP) [EPA 1975, 1978] has proposed, and the Organization for Economic Cooperation and Development (OECD 1981) is using K_{ow} as a measure of bioconcentration potential in aquatic organisms.

B. Rationale for the Selection of the Test Method

1. The Conventional Method of Determining the Octanol/
Water Partition Coefficient K_{ow}

The conventional method for determining a distribution coefficient is carried out by distributing a chemical between two immiscible liquids in a vessel and measuring the concentration of the chemical in the two liquid phases after equilibration (Glasstone 1946; Leo et al. 1971). This method can be applied to the determination of the octanol/water partition coefficient K_{ow} . Numerous researchers use the conventional method of determining K_{ow} and have published papers using this method (e.g., Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971; Chiou et al. 1977). EPA (EPA 1975, 1978) has proposed, and OECD (1981) is using the conventional method of determining K_{ow} . Most chemical companies that determine octanol/water partitioning use the conventional method of determining K_{ow} . Hence, the test guideline uses the conventional method of determining the octanol/water partition coefficient K_{ow} . It should be noted that there is no validated standard test method for determining K_{ow} (e.g., an ASTM method). The method in this guideline was

developed from a thorough review of the research literature on the experimental determination of K_{OW} and by talking to researchers who have considerable experience in carrying out these experiments.

2. Other Experimental Methods of Determining K_{OW}

a. Reverse-Phase High-Pressure Liquid Chromatography as a Method of Estimating K_{OW}

A rapid method based on reverse-phase high-pressure liquid chromatography has been developed by Veith (Veith and Morris 1978; Veith et al. 1979) to estimate the octanol/water partition coefficient of organic chemicals. Using the solvent mixture water/methanol (15/85 v/v) as the elutant, the log of the retention time [$\log(t_R)$] of organic chemicals on a permanently bonded (C-18) reverse-phase high-pressure liquid chromatographic system has been found to be linearly related to $\log K_{OW}$. This relationship has been expressed by the equation

$$\log K_{OW} = A \log(t_R) - B, \quad (1)$$

where A and B are constants determined from the experimental data for some organic chemicals. Using a mixture of the chemicals benzene, bromobenzene, biphenyl, p,p'-DDE [2,2-bis(p-chlorophenyl)-1,1-dichloroethylene] and 2,4,5,2',5'-pentachlorobiphenyl, A and B were found to be 5.106 and 1.258, respectively, with a coefficient

of determination of 0.975. It must be emphasized that this correlation is limited with respect to being representative of the organic chemicals encountered. This calibration mixture was selected largely on the basis of the $\log K_{OW}$ values reported in the literature, and the correlation is linear over five orders of magnitude of K_{OW} . To determine the accuracy of this method of estimating $\log K_{OW}$ by comparison with data reported in the literature, Veith and coworkers measured the retention time of 18 chemicals, and the standards and $\log K_{OW}$ values were calculated from the regression equation (1). The results indicated that $\log K_{OW}$ can be estimated to within (22.8 ± 20.0) percent when compared with the values reported in the literature from measurements using other methods. The percent error was calculated assuming the literature value is the correct $\log K_{OW}$; these researchers had some reservations about this assumption. It should be noted that some of the greatest relative errors were observed with polar chemicals that dissociate in water (e.g., m-chlorobenzoic acid, 2,4,5-trichlorophenol, and diphenylamine). This method has a definite advantage, since the estimation of K_{OW} can be made rapidly and relatively easily in comparison to the determination of K_{OW} by the conventional method. Furthermore, K_{OW} can be estimated for individual chemicals in complex mixtures (e.g., solid wastes) without knowing the specific chemical structure of each chemical.

Other researchers have developed high-pressure liquid chromatographic methods to determine K_{OW} (Mirrless et al. 1976; Yamana et al. 1977; Carlson et al. 1975; Hulshoff and Perrin 1976; McCall 1975). However, these methods are based on a very limited number of experiments and considerably more work is needed to develop them.

b. Thin-Layer Chromatography as a Method of Estimating K_{OW}

It has been reported that thin-layer chromatography can be used to estimate K_{OW} (Mirrless et al. 1976; Hulshoff and Perrin 1976). However, high-pressure liquid chromatography (HPLC) is far superior to thin-layer chromatography (TLC) because of its accuracy (i.e., definition of the peak, reproducibility, ease of detection in many cases, and above all the range of applicability (HPLC is applicable over 5 orders of magnitude of K_{OW} while TLC is only applicable over 1.5 orders of magnitude of K_{OW}) (Mirrless et al. 1976).

c. Estimation of K_{OW} from Water Solubility Data

The octanol/water partition coefficient is defined as the ratio of the equilibrium molar concentration of the chemical in octanol and water. Thus, low molecular mass (i.e., molecular weight) organic chemicals with a low water solubility should have

a high value of K_{OW} (e.g., hydrophobic organic chemicals). Therefore, there should be a correlation between K_{OW} and water solubility. Chiou et al. (1977) studied the relationship between K_{OW} and the water solubility, S , and found that, for 34 organic chemicals, an excellent linear correlation was observed between $\log K_{OW}$ and $\log S$ that extended to more than eight orders of magnitude in water solubility (10^{-3} to 10^4 ppm), and six orders of magnitude in K_{OW} (10 to 10^7). Chiou et al. (1977) found the following regression equation

$$\log K_{OW} = 5.00 - 0.670 \log S, \quad (2)$$

where K_{OW} is the octanol/water partition coefficient S is the water solubility in mol/L, and the coefficient of determination (r^2) was 0.970 for these 34 chemicals. Thus, K_{OW} can be estimated from the experimental value of the water solubility of an organic chemical. This method would have a definite advantage in that K_{OW} could be estimated directly from water solubility data without having to experimentally measure K_{OW} . Thus, the octanol/water test guideline could eventually be eliminated, thereby reducing the cost of testing. However, considerably more experimental work is necessary to extend the correlation to a large number of organic chemicals with different structures before it can be used as a test guideline.

C. Rationale for the Selection of Test Conditions

A detailed study of the theory of the distribution law, the partition coefficient, and the published literature on the conventional determination of K_{ow} indicates that it is extremely important that numerous factors (or test conditions) be standardized. In order to establish these factors clearly, the theory of the distribution law and its relation to these factors are discussed in detail in the following sections.

1. Theory of the Distribution Law and the Octanol/Water Partition Coefficient

The distribution coefficient or partition coefficient can be derived using thermodynamic theory (Glasstone 1946). Consider a mixture of two immiscible liquids that is shaken with a solute (organic chemical). The solute distributes itself between the two liquids in such a way that at equilibrium, in dilute solution, the ratio of the concentrations of the solute in the two layers is a constant at a given temperature. The tendency of a chemical to distribute itself between two immiscible liquids with a constant concentration ratio, in dilute solution, is a direct consequence of the thermodynamic requirements for equilibrium. To illustrate this, consider a pair of immiscible liquids A and B in contact with each other containing the same solute in solution. The chemical potential of a solute in solvent A is given by

$$\mu_A = \mu_A^0 + RT \ln a_A \quad , \quad (3)$$

where μ_A is the chemical potential of the solute in solvent A, μ_A^0 is the standard chemical potential of the solute in the same solvent (i.e., the value of μ_A at $a_A = 1$), while a_A , the activity of the solute in the solvent A is the effective concentration taking into account intermolecular interactions of the solute in the solvent. R is the gas constant and is equal to 8.314 joules/°K/mol, while T is the absolute temperature in °K. Similarly, for solvent B

$$\mu_B = \mu_B^0 + RT \ln a_B ,$$

where all the quantities have the same significance as in equation (3). At equilibrium between the layers $\Delta\mu = 0$; hence

$$\Delta\mu = \mu_B - \mu_A = 0 ,$$

and

$$\mu_B = \mu_A . \quad (5)$$

Using equations (3) and (4) in (5) yields

$$\mu_B^0 + RT \ln a_B = \mu_A^0 + RT \ln a_A \quad (6)$$

$$\ln \frac{a_B}{a_A} = \frac{\mu_A^0 - \mu_B^0}{RT}$$

However, at a given temperature, μ_B^0 and μ_A^0 are constants for a given solute in a particular solvent; hence

$$\ln \frac{a_B}{a_A} = K ,$$

and

$$\frac{a_B}{a_A} = K . \quad (7)$$

Equation (7) is the mathematical statement of the distribution law that states that a substance will distribute itself between two solvents until at equilibrium the ratio of the activities of a chemical in the two layers is a constant at a fixed temperature, irrespective of the absolute values of a_A or a_B . The activity a_A can be written as

$$a_A = \gamma_A C_A , \quad (8)$$

where γ_A is the activity coefficient and takes into account the interaction between molecules A in solution, and C_A is the molar concentration. In dilute solution as

$$C_A \rightarrow 0 ,$$

$$\gamma_A \rightarrow 1 ; \quad (9)$$

hence,

$$\lim_{C_A \rightarrow 0} (a_A) = C_A .$$

The same argument follows for the solute in solvents B and $\lim_{C_B \rightarrow 0} (a_B) = C_B$. Using these results in equation (7), the distribution coefficient K, in dilute solution, becomes

$$K = \lim_{C \rightarrow 0} \left(\frac{C_B}{C_A} \right) \quad (11)$$

For the specific case for the octanol/water partition coefficient, B is the solvent n-octanol, A is the solvent water, and $K^\circ = K_{ow}$. Thus, equation (11) becomes

$$K_{ow} = \frac{C_{\text{octanol}}}{C_{\text{water}}} \quad (12)$$

According to Nernst (1891), the distribution law applies only to individual molecular species in solution. If a molecule dissociates or associates in octanol and water, then equation (12) must be modified. In general, if α represents the fraction of the total solute that is dissociated or associated, assuming that either association or dissociation occurs in each solvent, then

$$K_{ow} = \frac{(1 - \alpha_{\text{oct.}}) C_{\text{oct.}}}{(1 - \alpha_{\text{water}}) C_{\text{water}}} \quad (13)$$

since $(1 - \alpha)$ gives the fraction of unchanged molecules in each phase. For the special case where no association takes place in octanol, equation (13) reduces to

$$K_{ow} = \frac{C_{\text{oct.}}}{(1 - \alpha_{\text{water}}) C_{\text{water}}} \quad (14)$$

2. Factors that Affect the Value of K_{ow}

a. Effect of Temperature.

From the theory of the distribution law as outlined in Section II.C.1, the distribution coefficient K is a function of the temperature (equation (6)), and is a constant at a fixed temperature (equation (7)). Since K_{ow} is a distribution coefficient, it should also vary with temperature and is a constant at a fixed temperature. Hence, in carrying out octanol/water partition coefficient experiments by the conventional method, the temperature should be controlled. However, variations due to temperature are small compared to those inherent in the errors in the other measurements, e.g., the errors in measuring the concentration of solute in octanol and water. Therefore, for reasonably accurate determinations of K_{ow} , it is sufficient to control the temperature to $\pm 1^\circ\text{C}$. Since most physical properties of chemicals are reported at 25°C , this guideline requires that K_{ow} be determined at this temperature.

b. Purity of the Solvents

Trace amounts of impurities present in n-octanol tend to produce emulsions and must be removed (Fujita et al. 1964; Hansch and Anderson 1967; Chiou et al. 1977). Emulsions give poor phase separation and result in a wide scatter in the value of K_{ow} . In addition, impurities in octanol may affect the analysis for the solute. Hence it is extremely important that the octanol be

99.9 percent pure. Distilled or reagent grade water (ASTM Type II) should be used.

c. Concentration of Solute

From the theory of the distribution law, as outlined in Section II.C.1, equations (12), (13), and (14) only apply in dilute solution. Hence, it is extremely important that all experiments be carried out at molar concentration $C \leq 0.01M$ in octanol and water.

d. Equilibration Time

For many chemicals, 5 minutes of gentle agitation of the two-phase system established equilibrium and produced consistent results (Leo et al. 1971). Studies by Craig and Craig (1950) indicated that when the phases were of about equal volume, equilibrium was rapidly attained. When high ratios of water to octanol (100:1) were used, longer shaking was necessary to establish equilibrium. High ratios of water to octanol are used to determine K_{ow} for very hydrophobic organic chemicals (Sections II.C.1.e and h as described below). Therefore, for most chemicals, gentle agitation for 1 hour should be adequate to reach equilibrium. For surfactants, at least 16 hours of agitation is necessary to reach equilibrium. This is an empirical observation obtained by researchers who have carried out experiments with surfactants. It is undoubtedly due to the nature of surfactant chemicals.

e. Octanol/Water Volume Ratio

Depending upon the solubility of the solute in octanol and water, the ratio of the volume of octanol to water should be adjusted. For hydrophobic solutes, which are very insoluble in water, considerably more water than octanol should be used. Adjustment of solvent volumes can decrease the effect of analytical errors and consequently decrease the error in determining K_{OW} (Leo et al. 1971). The following example will illustrate this point. Consider a chemical with a molecular mass (i.e., molecular weight) MW (mg/mmol), $K_{OW} = 200$. Twenty mg of chemical are dissolved in 100 mL of octanol, and 100 mL of water are added to this system. After equilibration, the mass of chemical in each phase can be calculated as follows. The chemical will partition with x mg in the water phase and (20-x) mg in the octanol phase.

$$C_{Oct.} = \frac{(20-x) \text{ mg}}{\frac{MW \text{ (mg/mmol)}}{100 \text{ mL}}} = \frac{(20-x)}{100 \text{ MW}} \frac{\text{mmol}}{\text{mL}}$$

$$= \frac{(20-x)}{100 \text{ MW}} \text{ Molar}$$

$$C_{water} = \frac{\frac{x \text{ mg}}{MW \text{ (mg/mmol)}}}{100 \text{ mL}} = \frac{x}{100 \text{ MW}} \frac{\text{mmol}}{\text{mL}}$$

$$= \frac{x}{100 \text{ MW}} \text{ Molar}$$

Since

$$K_{ow} = \frac{C_{oct.}}{C_{water}}$$

$$200 = \frac{\frac{(20-x)}{100 \text{ MW}}}{\frac{x}{100 \text{ MW}}} = \frac{(20-x)}{x}$$

$$\text{Then } x = \frac{20}{201} = 0.0996 = 0.10 \text{ mg} = \text{mass of chemical in the water phase;}$$

and

$$(20-x) = 19.9 \approx 20 \text{ mg} = \text{mass chemical in the octanol phase.}$$

Consider an analytical error of ± 0.05 mg in the aqueous phase (i.e., $0.10 - 0.05 = 0.05$ and $0.10 + 0.05 = 0.15$).

$$K_{ow} = \frac{\frac{\frac{20}{\text{MW}}}{100}}{\frac{0.05}{\frac{\text{MW}}{100}}} = \frac{20}{0.05} = 400$$

$$K_{ow} = \frac{\frac{\frac{20}{\text{MW}}}{100}}{\frac{0.15}{\frac{\text{MW}}{100}}} = \frac{20}{0.15} = 133.$$

Therefore, an analytical error of = 0.05 mg in the aqueous phase means that K_{ow} can range from 400 to 133, and a very large error in K_{ow} occurs.

Consider the same example as above, but now the solvents are adjusted to 200 mL of water and 5 mL of octanol. After equilibration, the mass of chemical in each phase is now:

$$C_{oct.} = \frac{\frac{(20-x)}{MW}}{5} = \frac{(20-x)}{5 MW} \text{ Molar}$$

$$C_{H_2O} = \frac{\frac{x}{MW}}{200} = \frac{x}{200 MW} \text{ Molar}$$

$$K_{ow} = \frac{C_{oct.}}{C_{water}}$$

$$200 = \frac{\frac{(20-x)}{5 MW}}{\frac{x}{200 MW}} = \frac{(20-x)(40)}{x}$$

Then

$$x = \frac{20}{6} = 3.33 \text{ mg} = \text{mass of chemical in the water phase}$$

and

$$(20-x) = 16.7 \text{ mg} = \text{mass of chemical in the octanol phase.}$$

Now consider the same analytical error of ± 0.05 mg in the aqueous phase (i.e., $3.33 - 0.05 = 3.28$ and $3.33 + 0.05 = 3.38$)

$$K_{ow} = \frac{\frac{\frac{16.7}{MW}}{5}}{\frac{\frac{3.28}{MW}}{200}} = \frac{16.7}{3.38}(40) = 203$$

$$K_{ow} = \frac{\frac{\frac{16.7}{MW}}{5}}{\frac{\frac{3.28}{MW}}{200}} = \frac{16.7}{3.28}(40) = 197 .$$

Now the analytical error of ± 0.05 mg in the aqueous phase means that K_{ow} can range from 197 to 203 and the error in K_{ow} has been reduced dramatically.

f. Chemical Analysis of the Octanol and Water Phases

Consider a partitioning experiment in which a chemical is dissolved in octanol at a low concentration (less than 0.01 molar). The conventional partitioning experiment is carried out, and only one phase is analyzed for the molar concentration of the solute. Using a mass balance, the molar concentration of the solute in the other phase is obtained by difference. However, if there is a loss of chemical by adsorption to the surface of the glass walls, a serious error will occur at this low concentration. This is especially true for very hydrophobic chemicals (Chiou et al. 1977) and for ionic solutes (Leo et al. 1971). Therefore, it is important that both the octanol and water phases be analyzed.

An analytical method should be selected that is the most applicable to the analysis of the specific chemical. However, large errors can occur as a result of traces of more-water-soluble contaminants that are not analytically distinguishable from the parent chemical. This error is very significant when the analytical method is ultraviolet absorption spectroscopy or radiometry, since these methods can be nonspecific for many solutes. Therefore, chromatographic methods are preferable because of their compound specificity in analyzing the parent chemical without interference from impurities (Karickhoff and Brown 1979). Whenever practicable, the chosen analytical method should have a precision with ± 5 percent.

g. Emulsification and Ultracentrifugation

Many chemicals can cause troublesome emulsions to form between octanol and water and emulsification can result in large errors in K_{ow} (Leo et al. 1971; Chiou et al. 1977). This is especially true for hydrophobic chemicals. Therefore, it is important that gentle shaking be used to minimize the formation of emulsions. In addition, incomplete separation of the two phases is one of the most serious sources of error. To break any emulsion formed and to separate completely the octanol and water phases, it is extremely important that the two-phase system be ultracentrifuged at 25°C for 20 minutes. The acceleration G value required to break an emulsion and to separate completely the octanol and water phases can be determined by trial-and-error experimentation. Since the visual clarity of the two phases is

not a dependable criterion of the absence of an emulsion and complete separation of the two-phase system, it is recommended that a turbidimeter be used to make sure that the emulsion is broken and the octanol and water phases have been completely separated.

h. Equilibration Vessel

To simplify the experimental procedure, it is recommended that equilibration be carried out in a centrifuge tube (special glass tubes can be used up to approximately 12,000 G and stainless steel centrifuge tubes can be used at higher G values) with a sealable cap. This will avoid a transfer step and volatile chemicals can be handled easily. It is important that the centrifuge tube be almost completely filled with the two-phase mixture to minimize partitioning with air. This is especially important when determining K_{ow} for volatile chemicals (Hansch and Anderson 1967).

Very hydrophobic chemicals, with K_{ow} on the order of 10^4 to 10^6 , require relatively large volumes of the aqueous phase (Section II.C.2.e). Hence, for these chemicals, it is recommended that equilibration be carried out in a large ground-glass stoppered flask.

i. Speciation Effects

The details of speciation have been discussed in the theory of the distribution law and the octanol/water partition coefficient, Section II.C.1.

If the chemical does not associate or dissociate in octanol and water, then the test guideline requires that equation (12) be used and K_{ow} be determined at concentrations $C \leq 0.01M$ and $C_1 = 0.1C$. Under these experimental conditions, if K_{ow} is constant, then association or dissociation has been minimized or eliminated.

If the chemical associates in octanol or water or in both liquids, then the test guideline requires that equation (13) be used and K_{ow} be determined at concentrations $C \leq 0.01M$, $C_1 = 0.1C$, $C_2 = 0.01C$, $C_3 = 0.001C, \dots$. When K_{ow} is constant at two concentrations differing by a factor of 10, then the effect of association has been minimized or eliminated.

For chemicals that reversibly ionize or protonate (e.g., carboxylic acids, phenols or anilines), the test guideline requires that equation (14) be used with water buffered at pH 5.0, 7.0, and 9.0, the pHs of environmental concern.

j. Presaturation of the Solvents

Presaturation of octanol with water and water with octanol is required for this test guideline. The preparation of these saturated solutions is very simple to carry out. This requirement

is extremely important when determining K_{OW} for very hydrophobic chemicals, since the ratio of water to octanol will be very large. In this case, if the experiment is carried out without presaturation of the water with octanol, then all the octanol will dissolve in the aqueous phase and K_{OW} cannot be determined. ■ ■ .

D. Reference Compounds

It would be very desirable to have reference compounds that cover a K_{OW} range of 10 to 10^6 . These reference compounds would provide the experimenter with comparative reference values to determine how well the test has been conducted. Unfortunately, these reference compounds are not currently available. When appropriate reference compounds have been identified they will be recommended for use in this test guideline. In the interim, it is recommended that the book by Hansch and Leo (1979) be used for the selection of potential reference compounds.

E. Test Data Required

The tendency of an organic chemical to partition out of water into other environmental compartments containing hydrophobic constituents (e.g., aquatic organisms) can be inferred from the values of the octanol/water partition coefficient K_{OW} . Chiou et al. (1977) developed regression equations relating $\log K_{OW}$ with water solubility S (in mol/L) and bioconcentration in rainbow trout (BF). Assuming $\log K_{OW}$ is between 1 and 6, S and BF can be

calculated; these results are summarized in Table 1 (note that S has been converted to mol/L). Furthermore, assuming that the average molecular mass (i.e., molecular weight) of an organic chemical is 300 gm/mol, the water solubility can be converted to ppm; these results are also summarized in Table 1. It is apparent that for $\log K_{OW} = 6$ (i.e., $K_{OW} = 10^6$), the water solubility will be extremely low (9.7×10^{-3} ppm or 9.7 ppb) and the predicted BF is 1.48×10^4 . Hence, the data indicate that the chemical will partition out of the water phase and into the fat of the fish (i.e., the hydrophobic phase). For $\log K_{OW} = 1$ (i.e., $K_{OW} = 10$), the water solubility will be very high (2.80×10^5 mg/L or 280 gm/L) and the predicted BF is 2.4. Hence, these data indicate that the chemical will remain in the water phase and will not partition significantly into the fat of the fish (i.e., the hydrophobic phase). Therefore, the test guideline is designed to determine the value of K_{OW} in the range 10 to 10^6 . Low molecular mass organic chemicals with a K_{OW} value less than 10 will not partition significantly into or tend to accumulate in, any hydrophobic environmental compartments. Low molecular mass organic chemicals with K_{OW} in excess of 10^6 will tend to accumulate into all hydrophobic environmental compartments. For low molecular mass organic chemicals outside the range 10 to 10^6 , the test guideline requires that K_{OW} be characterized as 10 or 10^6 with no further quantification.

Specific analytical and recovery procedures should be reported to determine whether acceptable data have been generated.

Table 1. Summary of Calculated Values of Water Solubility and Bioconcentration in Rainbow Trout as a Function of Log K_{ow} ^a

log K_{ow}	S(mol/L) ^b	S(mg/L or ppm) ^c	BF ^d
6	3.24×10^{-8}	9.7×10^{-3}	1.48×10^4
5	1×10^{-6}	0.30	2.57×10^3
4	3.09×10^{-5}	9.3	4.47×10^2
3	9.77×10^{-4}	2.93×10^2	7.76×10^1
2	3.02×10^{-2}	9.06×10^3	1.35×10^1
1	9.3×10^{-1}	2.80×10^5	2.4

^aRegression equations taken from Chiou et al. (1977).

^bWater solubility

^cWater solubility in ppm assuming a molecular mass of an organic chemical is 300 gm/mol.

^dBioconcentration in rainbow trout

F. Statistical Analysis of the Data

Numerous researchers have published data on the determination of the octanol/water partition coefficient by the conventional method (e.g., Chiou et al. 1977; Davies et al. 1975; Fujita et al. 1964; Hansch and Anderson 1967; Leo et al. 1971). However, none of these researchers has analyzed the data statistically and the precision of K_{OW} as determined by the conventional method has not been clearly established. The precision is, in part, a function of the nature of the specific chemical. As the hydrophobicity of the chemical increases, K_{OW} increases and the precision of K_{OW} decreases. Furthermore, the precision is also a function of the analytical procedure used. In general, the lower the concentration to be measured, the poorer is the precision of the analytical procedure. Therefore, no reliable precision can be stated at this time for determining K_{OW} . Obviously, the precision can be improved by making numerous replicate determinations. However, in order to minimize cost, it has been decided to determine K_{OW} with three replicates. Therefore, it is important that the submitter of the test results analyze the data statistically. When a large number of chemicals have been determined by the proposed method, the data will be analyzed statistically and the level of precision can be defined for various ranges of K_{OW} .

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WATER SOLUBILITY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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WATER SOLUBILITYI. INTRODUCTIONA. Background and Purpose

The water solubility of a compound can be defined as the equilibrium concentration of the compound in a saturated aqueous solution at a given temperature and pressure. The water solubility of a chemical is an important factor in determining the environmental movement and distribution of any substance. Chemicals that are relatively water soluble are more likely to be widely distributed by the hydrologic cycle than those which are relatively insoluble.

Water provides the medium in which many organisms live, and water is a major component of the internal environment of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most other chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

The water solubility of a chemical has an effect on its adsorption on and desorption from soils and sediments and on volatilization from aqueous media. The more soluble a chemical substance is, the more likely it is to desorb from soils and

sediments and the less likely it is to volatilize from water. The extent of chemical transformations via hydrolysis, photolysis, oxidation, reduction, and biodegradation in water depends on the chemical being soluble in water (i.e., homogeneous kinetics). Finally, the design of most chemical tests and many ecological and health tests requires precise knowledge of the water solubility of the chemical to be tested.

Procedures in this test guideline have been described to enable sponsors to determine the water solubility for solid and liquid organic compounds.

B. Definitions and Units

(1) "Colloidal dispersion" is a mixture resembling a true solution but containing one or more substances that are finely divided but large enough to prevent passage through a semipermeable membrane. It consists of particles which are larger than molecules, which settle out very slowly with time, which scatter a beam of light, and which are too small for resolution with an ordinary light microscope.

(2) A "concentration vs. time study" results in a graph which plots the measured concentration of a given compound in a solution as a function of elapsed time. Usually, it provides a more reliable determination of equilibrium water solubility of hydrophobic compounds than can be obtained by single measurements of separate samples.

(3) "Concentration" of a solution is the amount of solute in a given amount of solvent and can be expressed as a weight/weight or weight/volume relationship. The conversion from a weight relationship to one of volume incorporates density as a factor. For dilute aqueous solutions, the density of the solvent is approximately equal to the density of the solutions; thus, concentrations in mg/dm^3 are approximately equal to $10^{-3} \text{ g/10}^3 \text{ g}$ or parts per million (ppm); ones in $\mu\text{g/dm}^3$ are approximately equal to $10^{-6} \text{ g/10}^3 \text{ g}$ or parts per billion (ppb). In addition, concentration can be expressed in terms of molarity, normality, molality, and mole fraction. For example, to convert from weight/volume to molarity one incorporates molecular mass as a factor.

(4) "Density" is the mass of a unit volume of a material. It is a function of temperature, hence the temperature at which it is measured should be specified. For a solid, it is the density of the impermeable portion rather than the bulk density. For solids and liquids, suitable units of measurement are g/cm^3 . The density of a solution is the mass of a unit volume of the solution and suitable units of measurement are g/cm^3 .

(5) An "oversaturated (supersaturated) solution" is a solution that contains a greater concentration of a solute than is possible at equilibrium under fixed conditions of temperature and pressure.

(6) A "saturated solution" is a solution in which the dissolved solute is in equilibrium with an excess of undissolved solute; or a solution in equilibrium such that at a fixed temperature and pressure, the concentration of the solution is at its maximum value and will not change even in the presence of an excess of solute.

(7) A "solution" is a homogeneous mixture of two or more substances constituting a single phase.

C. Principle of the Test Method

The test method is based on the conventional method of preparing saturated aqueous solutions. The method involves the coating of the compound to the walls of a vessel, adding water (i.e., very pure water, buffer solution, or artificial seawater), and determining the concentration of the compound in the water as a function of time at a fixed temperature. When the concentration reaches a plateau, equilibrium has been achieved, and the water is saturated with the compound. Specific procedures have been incorporated in this test guideline to measure the water solubility of very hydrophobic compounds and to alleviate the problems of colloids and emulsions usually formed.

D. Applicability and Specificity

Procedures have been described in this test guideline to determine the saturated water solubility for liquid or solid compounds. The water solubility can be determined in very pure

water, buffer solution for compounds that reversibly ionize or protonate, or in artificial seawater as a function of temperature (i.e., in the range of temperatures of environmental concern). Water solubility is usually not useful for gases because their solubility in water is measured when the gas above the water is at a partial pressure of one atmosphere which is several orders of magnitude greater than those existing under environmental conditions. A more important parameter for gases is Henry's law constant which is the ratio of the vapor pressure of the compound to solution concentration at low partial pressures.

This test guideline is designed to determine the saturated water solubility of a solid or liquid test chemical in the range infinity to 10 parts per billion (ppb). For chemicals whose solubility is below 10 ppb, the water solubility should be characterized as "less than 10 ppb" with no further quantification.

II. DESCRIPTION OF THE TEST PROCEDURE

A. Test Conditions

1. Special Laboratory Equipment

- (1) A thermostatic bath with temperature control ($\pm 1^{\circ}\text{C}$) in the approximate range of 5-30°C;
- (2) an ultracentrifuge with temperature control ($\pm 1^{\circ}\text{C}$) in the approximate range of 5-30°C and capable of obtaining acceleration (G) values to 39,000 or higher;

- (3) a pH meter capable of resolving differences of 0.1 pH units or less; and
- (4) centrifuge tubes with sealable caps: special glass tubes can be used up to approximately 12,000 G; tubes to be used at G value > 12,000 should be made of stainless steel.

2. Purity of Water

Reagent grade water, e.g., water meeting ASTM Type IIA standards or an equivalent grade, is highly recommended to minimize biodegradation and to minimize the effects of dissolved salts on water solubility. ASTM Type IIA water is described in ASTM D1193-77, "Standard Specification for Reagent Water".

3. Purity of Solvents

It is important that all solvents used for coating test compounds on the walls of vessels and in separation and analytical technique be reagent grade and contain no impurities which will interfere with the determination of the test compound.

4. Seawater

It is recommended that artificial seawater be used to determine the saturated water solubility in seawater. The preparation of artificial seawater is described in Section II.B.2.

5. Agitation and Equilibration Time

It is important that contact time of test compounds with water be sufficient to obtain a saturated solution. The length of time necessary will depend upon such variables as the size of the vessel, the extent and degree of agitation, the properties of the compound and particle size. To increase the rate of solution of hydrophobic compounds, a mild agitation is recommended. For hydrophobic compounds a minimum time of one day is required.

6. Effects of Colloids and Emulsions: Centrifugation

It is important that gentle shaking be used to minimize the formation of colloids. The presence of colloids and emulsions will lead to solubility values that are higher than those in a true saturated solution. This is a common problem with hydrophobic solids and liquids but can usually be overcome by centrifugation. It is recommended that centrifugation be conducted in tightly sealed tubes that are almost filled to capacity to avoid partitioning with air.

It is extremely important that centrifugation be carried out at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25°C until concentration changes are small. For hydrophobic compounds (solubility ≤ 10 ppm), it is extremely important that the acceleration G values differ by 10,000 G and include a determination of 39,000 or higher.

7. Effect of pH on Solubility

It is recommended that all experiments be carried out at pH's 5.0, 7.0, and 9.0 for any chemical which reversibly ionizes or protonates (e.g., carboxylic acids, phenols, amines). Buffers described in Section II.B.2 can be used.

8. Analysis of Saturated Solutions

Any suitable analytical method may be used; where practicable, precision should be within ± 5 percent. Preferred analytical methods are those that are specific for the compound to be tested, to the exclusion of other compounds. Chromatographic methods which incorporate separation, and therefore, specification, are recommended.

9. Adsorption to Glass or Other Surfaces

Hydrophobic compounds have a tendency to adsorb to glass or other surfaces, e.g., stainless steel. Thus, when transferring the solution to any glass vessel or container, it is essential to pre-rinse the surfaces of the vessel or container with the solution. Failure to do so will lead to solubility values that are lower than those of true equilibrium water solubility because the compound will adsorb to the unrinsed surface. However, when hydrophobic compounds are extracted with organic solvent, the extraction vessels should not be pre-rinsed since this would lead to solubility values that are greater than those of true equilibrium water solubility.

B. Preparation of Reagents and Solutions

1. Buffer Solutions

Prepare buffer solutions using reagent grade water as follows:

- pH 5.0-- To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.
- pH 7.0-- To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent gradewater.
- pH 9.0-- To 250 mL of 0.075M borax add 69 ml of 0.1M HCL; adjust the final volume to 500 mL with reagent gradewater.

Check the pH of each buffer solution with a pH meter at 25°C and adjust to pH 5.0, 7.0, or 9.0, if necessary. If the pH of the solution has changed by ± 0.2 pH units or more after the addition of the test compound, then a more concentrated buffer is required for that pH determination. The sponsor should then choose a more suitable buffer.

2. Artificial Seawater

Add the reagent-grade chemicals listed in Table 1 in the specified amounts and order to 890 mL of reagent-grade water. It is important that each chemical be dissolved before another one is added.

C. Performance of the Test

Determine the saturated water solubility of the test compound at 25°C in reagent grade water or buffer solution, if appropriate. Under certain circumstances, it may be necessary to determine the water solubility of a test compound at 25°C in artificial seawater. The water solubility can also be determined at other temperatures of environmental concern by adjusting the temperature of the water bath to the appropriate temperature.

1. Procedure for the Determination of Solids and Liquids in Water at 25°C

Dissolve a sufficient amount of the solid compound in a suitable volatile organic solvent and coat on the walls of a vessel. Viscous liquids may be coated on vessels in a similar fashion; non-viscous liquids do not require solvents. Remove the solvent under reduced pressure or with a pure nitrogen gas stream. When all the solvent is removed, add reagent grade water or, for compounds which reversibly ionize or protonate, the appropriate buffer solution and slowly stir or agitate the mixture under temperature control. Mixing may be accomplished by

Table 1--Constituents of Artificial Seawater¹

Chemical	Amount
NaF	3 mg
SrCl ₂ ·6H ₂ O	20 mg
H ₃ BO ₃	30 mg
KBr	1000 mg
KCl	700 mg
CaCl ₂ ·2H ₂ O	1.47 g
Na ₂ SO ₄	4.00 g
MgCl ₂ ·6H ₂ O	10.78 g
NaCl	23.50 g
Na ₂ SiO ₃ ·9H ₂ O	20 mg
NaHCO ₃	200 mg

¹If the resulting solution is diluted to 1 cubic decimeter (1 liter), the salinity should be 34 ± 0.5 g/kg and the pH 8.0 ± 0.2 . The desired test salinity is attained by dilution at time of use.

use of a Teflon coated stirring bar and should be continued for a minimum of 24 hours before aliquots are withdrawn. Prior to taking aliquots, the mixture should be left to stand at constant temperature for at least one hour to permit separation of any small particles. To determine the concentration of the compound in the aqueous phase, aliquots should be centrifuged at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25°C until concentration changes are small. The concentration value so obtained is plotted against the time of mixing. At a latter time, aliquots are again taken and analyzed in the same fashion to produce another data point on a concentration vs. time plot. When the concentration reaches a plateau, equilibrium is assumed. For hydrophobic compounds (solubility ≤ 10 ppm) it is extremely important that the acceleration (G) values differ by 10,000 G and include a determination at 39,000 G or higher.

For more soluble compounds (solubility ≥ 500 ppm) coating the walls of the vessel is not necessary and filtration may be substituted for centrifugation. Use filters which are adequate to remove suspended particles. If the concentration of the solute exceeds 10 g/dm^3 , then determine the density of the solution. This can be done by weighing known volumes of the solution at the same temperature as the constant temperature bath. Sufficient solution should be used so that each determination is made on a fresh aliquot. Carry out solubility and density experiments in triplicate.

2. Modification of Procedures for Potential Problems

a. Interference of Soluble Impurities

Interference by soluble impurities in the test sample can be avoided by the use of an analytical technique that is specific to the compound being tested. If this is not practical, interferences can sometimes be minimized by repeatedly preparing saturated solutions from the same sample chemical until the concentration of the impurity has been depleted.

b. Decomposition of the Test Compound

If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be obtained, then it will be necessary to carry out detailed transformation studies e.g., hydrolysis. If decomposition is due to aqueous photolysis, then it will be necessary to carry out water solubility studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

III. DATA AND REPORTING

A. Test Report

For each set of conditions, (e.g., temperature, pure water, buffer solution, artificial seawater) required for the study,

provide the water solubility value for each of three determinations, the mean value, and the standard deviation.

For compounds that decompose at a rate such that a precise value for the water solubility cannot be obtained, provide a statement to that effect.

For compounds with water solubility below 10 ppb, report the value as "less than 10 ppb."

For compounds with water solubility greater than 10 g/dm³, report the density of the solution at each required temperature.

B. Specific Analytical and Recovery Procedures

- (1) Provide a detailed description or references for the analytical procedure used, including the calibration data and precision; and
- (2) if extraction methods were used to separate the solute from the aqueous solution provide a description of the extraction method as well as the recovery data.

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1193-77.

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WATER SOLUBILITY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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WATER SOLUBILITYI. NEED FOR THE TEST

The water solubility of a compound can be defined as the equilibrium concentration of the compound in a saturated aqueous solution at a given temperature. The water solubility is a fundamental physical property of a compound. It is an essential characteristic for determining that compound's movement and distribution in the environment and, therefore, its potential effects on living organisms. Highly soluble compounds are more likely to be distributed by the hydrologic cycle than less soluble compounds. The degree of water solubility of a compound can affect its adsorption and desorption on soils and sediments and ease of volatility from aquatic systems. Substances which are more soluble in water are more likely to desorb from soils and sediments and less likely to volatilize from water. Transformations such as hydrolysis, photolysis, oxidation, reduction, and biodegradation in water proceed more rapidly when the compound is dissolved in water (i.e., homogeneous kinetics). The potential importance of these transformations is, therefore, a function of the ability of a compound to dissolve in water. The design of most chemical tests and many ecological and health tests requires accurate knowledge of the water solubility of the compound to be tested.

Virtually all modeling systems devised to determine the distribution of a compound in the environment require water solubility data. Although attempts have been made to correlate

water solubility with other physical parameters such as structure, enthalpy of fusion, melting point and molar volume, in order to predict or calculate the water solubility of a compound, it is generally agreed that an actual physical measurement of water solubility is a far more accurate and desirable approach (Mader and Grady 1970). In addition, values of water solubility have been shown to correlate with the octanol/water partition coefficient (K_{ow}) for a number of compounds (Chiou et al. 1977, Yalkowsky and Valvani 1979, 1980, 1981). These correlations enable a quite accurate estimate of a compound's K_{ow} to be made from its water solubility.

Water provides the medium in which many organisms live, and water is a major component of the internal portion of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

Water solubility is an essential parameter for assessing the environmental partitioning of all solid or liquid chemicals. Water solubility is usually not useful for gases because their solubility in water is measured when the gas above the water is at a partial pressure of one atmosphere which is several orders

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of magnitude greater than those existing under environmental conditions. Thus, water solubility of gases does not generally apply to environmental assessment because the actual partial pressure of a gas in the environment is extremely low.

II. SCIENTIFIC ASPECTS

A. Rationale for the Selection of the Test Method

Analytical methods for the determination of water solubility consist of obtaining an equilibrium saturated aqueous solution of the compound and analyzing the solution by some suitable physical or chemical method. Equilibrium may be obtained by intimately mixing the solute and solvent. Separation of the phases can then be accomplished by filtration or decantation, usually followed by centrifugation.

The solubility of a compound in water at equilibrium is a function of temperature, pressure, and purity of solute and water (Mader and Grady 1970). A major practical difficulty is determining that equilibrium has actually been attained. There are two general methods to determine the attainment of equilibrium. The concentration vs. time method (Mader and Grady 1970) involves periodic sampling over meaningful periods of time; when the concentration has reached a plateau, equilibrium has been attained. The undersaturation/oversaturation method (Mader and Grady 1970) refers to approaching equilibrium from both possible non-equilibrium conditions. One of two identical

solutions is heated to a temperature well above the required test temperature so that the solubility is increased. Then both solutions are placed in a thermostatic bath to equilibrate at the required test temperature. The heated solution becomes supersaturated (oversaturated) when its temperature decreases and reaches equilibrium as the concentration of the dissolved solute decreases to a plateau at the required test temperature. The other solution is undersaturated and reaches equilibrium as the concentration of dissolved solute increases to a plateau at the required test temperature. If identical concentrations in aliquots of these two samples are obtained after a period of time, equilibrium has been reached. Research sponsored by EPA at the Battelle Institute demonstrated that the under/oversaturation method will give poor analytical results if the compound tested undergoes a phase transition in the temperature range utilized (EPA 1981). Thus, a low melting solid may recrystallize at a very slow rate after being heated to elevated temperatures and make separation of excess solute from the aqueous phase extremely difficult. Other potential problems include accelerated decomposition of the test material at elevated temperatures and dramatically different changes in solubility rates of impurities vs. test compound with change in temperature.

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The method described in the test guideline involves coating the compound on the walls of a vessel, adding very pure water, and determining the change in concentration of the compound in the water over a period of time at a fixed temperature (Haque and Schmedding 1975, Karickhoff and Brown 1979). Typically, the

concentration of a compound dissolved in the aqueous phase will increase with time. As the solution progresses toward saturation, the rate of change of concentration will decrease and ultimately, the concentration will reach a plateau. The formation of that plateau after a suitable period of time has elapsed is generally considered an accurate indication that equilibrium has been established. The coating of solids on the walls of the vessel is considered to be more practical than suspending the solid as small particles in water since the coating technique minimizes the formation of microcrystals that are difficult to remove.

The use of a single procedure to cover the entire range of solubilities from infinity down to 10 ppb has the obvious advantage of not having to change procedures near the cut-off of two ranges. Sufficient flexibility has been incorporated into the test guideline to accommodate a wide range of solubilities.

The use of a "suitable solvent" for coating the vessel walls allows the sponsor to choose a solvent that is more appropriate for the test compound in recognition of the possible variation in the physical properties of different compounds. Typically, solvents such as acetone, methanol, ether, dichloromethane, and hexane can be employed.

It has been shown that constant but erroneous solubility values can be obtained when very small droplets or particles remain dispersed in water (Biggar and Riggs 1974). To separate

these colloidal particles, centrifugation (or ultracentrifugation) is necessary (Biggar and Riggs 1974). If constant concentration values are obtained after centrifugation at several different acceleration (G) values, one can assume that removal of colloidal particles has been achieved. The centrifuge tubes should be tightly sealed because the test compound can escape from the solution through volatilization, especially for those compounds with an appreciable vapor pressure. In addition, the tubes should be filled almost to capacity to minimize partitioning to air.

The less soluble the test compound, the greater the error introduced by the presence of suspended particles. For example, the suspension of $100 \mu\text{g}/\text{dm}^3$ of undissolved material is small if the true solubility is $100 \text{ mg}/\text{dm}^3$ (error of 0.1 percent) but is extremely large if the true solubility is $100 \mu\text{g}/\text{dm}^3$ (error of 100 percent). Thus, more extensive ultracentrifuge procedures will be required for more hydrophobic compounds. It has been demonstrated that reliable solubility determinations can be made for very hydrophobic chemicals by centrifuging the suspension at two or three different G values for one-half hour at constant temperature until concentration changes are small (Biggar and Riggs 1974). For several pesticides, centrifugation at 39,000 G removed the colloidal particles corresponding to $0.1 \mu\text{m}$ particle size. For DDT, it had been demonstrated that one hour of centrifugation at 84,000 G removed all the colloidal particles (Bowman, Acree, and Corbett 1960). For chemicals that are soluble in the range 500 to 10 ppm, aliquots of the suspension

must be centrifuged at two or three different G values for at least 30 minutes at constant temperature until concentration changes are small. For hydrophobic chemicals (solubility ≤ 10 ppm), it is extremely important that acceleration (G) values differ by 10,000 G and include a determination at 39,000 G or higher. When determining the solubility of relatively soluble compounds (500 ppm), large solid particles can be conveniently separated by filtration, and macropore filters may be employed as part of the separation technique.

It is recommended that the analytical technique be selected by the sponsor and/or testing laboratory in recognition of the many different techniques available and the advantage of being able to match one to the properties of the compound (e.g, the degree of solubility, the spectroscopic properties of the compound, and its chromatographic behavior). Analytical techniques that allow the quantitative determination of the test compound to the exclusion of impurities are recommended to the extent practicable. Therefore, chromatographic techniques are recommended.

B. Other Methods of Determining Water Solubility

1. Interferometry

This method has been used to determine the solubility of slightly soluble solids and liquids in water in the ppm range (Adams 1915, Mader and Grady 1970). Limited research has been

done on this method, and the technique has only been applied to a few compounds. It does not have general applicability.

2. Nephelometry

The nephelometric (turbidity) method calls for preparation of stable suspensions of the organic chemical at several different concentrations exceeding the nominal solubility. The turbidities of the resulting suspensions are then measured and plotted against total concentration. A Beer's Law relationship is assumed; thus, a straight line drawn through the points yields an intercept equal to the solubility, i.e., the concentration at which the turbidity vanishes (Davis and Parke 1942).

In tests of this method a complete suspension of excess solute could not be accomplished by mechanical means such as sonication. In order to obtain a complete suspension, the procedure requires that the compound be dissolved in a solvent that is miscible with water. Initial amounts of dissolved solute that are added tend to disperse in the water and form a true solution. However, as the solubility limit is approached, turbid zones are produced that persist briefly depending on the stirring efficiency, and a permanent turbidity is formed at the solubility limit.

In principle, this approach can be used to give a rough initial estimate of the solubility. The resulting solubility is subject to several conditions and limitations, including the

inaccuracy and lack of reproducibility of the individual turbidity measurements, the instability of the original particle size distribution (a function of time and dilution), and the effect of the dispersing agent (solvent) on the solubility. These conditions and limitations are not independent of each other. For example, the use of solvent pairs to form a liquid having solvent properties intermediate between those of the parent solvents is a well-recognized technique (e.g., as applied with gradient elution HPLC). Thus, not only is it to be expected that the use of a dispersing agent might influence the true solubility, but the solubility and hence the particle size distribution might be expected to change as a function of dilution of the stock solution. Also, in cases where the solubility is strongly influenced by the presence of the dispersing solvent, evaporative loss of the dispersing solvent can lead to continued precipitation of the solute and thus influence the particle size distribution and apparent turbidity.

The stability of the particle size distribution can also be affected by the nature of the solute. With a solute that has a relatively high mobility, it can be expected that the large particles will grow at the expense of the smaller particles thus altering the apparent turbidity. If the solute is volatile, an appreciable fraction of the solute may be lost from the suspension, and recrystallization may occur on the surface of the water or on other surfaces external to the bulk liquid.

Therefore, the nephelometric method has been rejected as a general method for determining the water solubility of organic compounds.

3. Coupled Column Liquid Chromatography

This is a recently published method used to determine the aqueous solubility of hydrophobic polycyclic aromatic hydrocarbons (May, Wasik, and Freeman 1978a,b). The method consists of pumping water through a column containing glass beads coated with the compound being studied. The beads are prepared by adding them to a 0.1 percent methylene chloride solution of the test compound and stripping the solvent with a rotary evaporator. The coated beads are packed into stainless steel tubes. Saturated solutions are generated by pumping water through these thermostated columns at flow rates ranging between 0.1 and 5 mL/min. These saturated solutions are extracted by flowing a measured volume of solution through a stainless steel extactor column where the material is adsorbed to a special column packing. Another solvent system is then used to elute the test material from the extactor column onto an analytical column where its concentration is measured by a standard liquid chromatography detector system. It is necessary to calibrate the detector signal by using solutions of known concentration. Precision in the ppb range is excellent (± 3 percent). The method appears promising but needs more experimental work on a variety of hydrophobic chemicals before it can be determined whether or not to recommend the procedure.

C. Rationale for the Selection of the
Test Conditions

1. Temperature

The equilibrium water solubility of a substance is a constant at a fixed temperature and pressure. For most solid and liquid compounds, an increase in temperature results in an increase in water solubility. The rate of change in water solubility with temperature is not linear for a given chemical and varies dramatically from one substance to another (Mader and Grady 1970). For many compounds the following relationship describes concentration (water solubility) as a function of temperature:

$$\ln \frac{1}{X_a} = \frac{\Delta H}{R} \left(\frac{1}{T} - \frac{1}{T_f} \right),$$

where X_a is the mole fraction of compound A, ΔH is the enthalpy of fusion, T_f is the thermodynamic temperature of fusion and R is the universal gas constant (Bigger and Riggs 1974). The equation can be put into the form $\log_{10} C = -\frac{A}{T} + B$, where C = molar concentration, T is the thermodynamic temperature and A and B are constants. By determining C as a function of T (e.g., at 5, 15, 25°C), the data can be used to determine A and B (i.e., plot

$\log_{10}C$ vs. $1/T$; the slope of the line is A and the intercept is B). Thus, one can use this equation to estimate C at any environmental temperature of concern. As a working estimate, scientists often anticipate a doubling of solubility with an increase of 10°C . The temperature chosen for this test guideline is 25°C since this is the temperature traditionally used for reporting physical properties. Solubility determinations may be requested at additional temperatures of environmental concern for specific compounds or classes of compounds.

Since water solubility values will change with temperature (Mader and Grady 1970), the temperature should be controlled to $\pm 1^{\circ}\text{C}$. This is a condition easily achieved by standard temperature control devices for water baths and is a practical range for temperature controlled centrifuges.

2. Purity of Water

Dissolved salts can affect the water solubility of a compound, necessitating the use of very pure water (Mader and Grady 1970). In addition, the water should be relatively free of bacteria which may consume or alter the organic test material during extended periods of testing. ASTM Type II reagent grade water (ASTM 1978), or an equivalent grade containing less than 1 mg/dm^3 total organic carbon, has been recommended for use in this test guideline to minimize biodegradation and the effects of impurities.

3. Purity of Solvents

Organic solvents that come into contact with the test material and aqueous solution must be as pure as practicable. Trace organic impurities that are soluble in water can alter the water solubility of the test material as well as interfere with the analysis of the concentration of the test material.

4. Seawater

Organic compounds are often released to seawater. The presence of dissolved salts will usually alter the true water solubility of a compound. Typically, the water solubility of an organic compound will be less in seawater than in pure water at a fixed temperature (Long and McDevit 1952). Data on solubility in seawater is needed to design ecological testing of marine species and for modeling. Salinity varies in different marine environments. In order to determine solubility under uniform conditions, a formulation for artificial seawater is specified in the test guideline (Kester et al. 1967, EPA 1975a).

5. Agitation and Equilibration Time

To increase the rate of solution of hydrophobic compounds, mild agitation is recommended. The use of strong agitation, while increasing the rate of solution, also increases the formation of emulsions and colloids by producing very small particles which remain suspended in solution. For hydrophobic

compounds, experience has shown that a minimum of one day with mild agitation is necessary to reach equilibrium, and several weeks are often required (Haque and Schmedding 1975).

6. Effects of Colloids and Emulsions: Centrifugation

The use of a centrifuge allows the rapid separation of small particles suspended in water. The size of the particles separated depends on the acceleration factor (G value) and the length of time the sample is centrifuged. Centrifugation at two or three different G values will partition suspended particles to two or three different distributions. When the water solubility value obtained is relatively constant at two different G values, this indicates that any suspended particles present are relatively insignificant (Biggar and Riggs 1974). It is important that centrifugation be conducted in tightly sealed tubes that are almost filled to capacity to avoid partitioning with air and loss of chemical via volatilization.

7. Effect of pH on Solubility

It is known that the water solubility of some organic compounds will alter with change in the pH of the solution (Cheung and Biggar 1974). This change will not be dramatic unless the compound contains readily ionizable or protonated groups. For carboxylic acids, amines and compounds which reversibly ionize or protonate in water, it is necessary to

measure the water solubility at pH values of 5.0, 7.0, and 9.0, since these pH values are representative of those found in natural aquatic ecosystems, groundwater, and rainwater.

The specified buffer solutions contain an adequate concentration of buffer to neutralize approximately 100-500 ppm of dissolved test compound (depending on its molecular weight) without a significant change in pH and, therefore, will be satisfactory for most test compounds. A change in pH of 0.2 units signifies that a more concentrated buffer solution is needed. In such cases, the choice of buffer solution should be made by the sponsor in order for it to be compatible with their analytical scheme.

8. Analysis of Saturated Solutions

The diversity of compounds to be tested precludes the specification of a limited number of analytical techniques; the choice is best left to the sponsor. A great many analytical procedures are potentially useful. Nonetheless, the use of a compound-specific analytical procedure has been recommended for use in this test guideline. Since chromatographic techniques entail separation as well as quantification for many organic compounds, these methods have been recommended. Where procedures are available, chromatographic techniques are cost effective. However, determining the proper chromatographic procedure can be time consuming. Depending upon the physical properties exhibited by the compound and its equilibrium concentration in water, other

standard techniques can be more efficient with respect to ease of analysis, precision, and cost. Whenever practicable, an analytical procedure should be used having a precision within ± 5 percent. Scientists are generally aware of the wide variety of standard techniques available today and of the large number of methods being developed, so that a discussion of even a small percentage of analytical possibilities would serve little purpose. This test guideline does require, however, that the specific analytical technique utilized be adequately described. Some procedures involve the use of extracting solvents and, when so used, it is important that these extraction procedures be adequately described and recovery information be submitted.

9. Adsorption to Glass or Other Surfaces

Hydrophobic compounds will have a tendency to adsorb to glass or other surfaces (e.g., stainless steel). Thus, when transferring the test solution to any vessel or container, it is essential to pre-rinse the surfaces with the solution. Failure to do so will lead to solubility values that are lower than those of true equilibrium water solubility. However, when hydrophobic compounds are extracted with organic solvent, their containers should not be pre-rinsed since this would lead to solubility values that are greater than those of true equilibrium water solubility.

D. Test Data Required

Present analytical techniques allow the determination of the concentration of organic compounds in water as low as the parts per billion range and, in time, this capability may be extended. However, using current techniques the reliability and precision below 10 ppb will be ± 50 percent at best. Because of these inherent inaccuracies, the greatly increased costs associated with quantification below 10 ppb would be difficult to justify. Furthermore, organic substances that are so hydrophobic that their water solubility is less than 10 ppb may disperse in water forming micelles rather than being truly soluble. Thus, for substances whose water solubility is below 10 ppb, the test guideline requires that the water solubility be characterized as "less than 10 ppb" with no further quantification.

When the solubility of a compound is equal to or greater than 10 g/dm^3 , it is important that the density of the saturated solution be determined experimentally and reported. For solutions of such concentration, density is needed to convert from a concentration of g/dm^3 to other units (e.g., ppm, mole fraction, molality).

E. Statistical Analysis of the Data

Numerous researchers have published measurements of water solubility using a variety of methods (Gunther et al. 1968). For many methods, good precision (i.e., repeatable values) can be obtained but the comparison of one method to another may give

larger differences than would be expected from the precision of each one. Therefore, though precise, the water solubility data may not be accurate (i.e., correct). For example, the water solubility of DDT has been investigated by many researchers. It was finally demonstrated that values obtained depended upon the size distribution of suspended particles in the "saturated aqueous solution". Suspended particle size distribution was a function of time of centrifuging and acceleration (G) values (Biggar and Riggs 1974). Therefore, significantly different values (each with good precision) could be obtained for the different sets of conditions. This problem is more significant for very hydrophobic compounds than for those which are more water soluble. It is one of the reasons why the method does not require quantification below 10 ppb.

The precision of the water solubility data generated by the proposed general method has not been clearly established. As the solubility decreases, the precision is expected to become poorer. The precision is also a function of the nature of the specific compound and the analytical procedure used. Therefore, no reliable precision can be stated at this time for determining the water solubility. Obviously, the precision can be improved by making numerous replicate determinations. However, in order to minimize costs, it has been decided to determine water

solubility with three replicates. Therefore, it is extremely important that the submitter of the test results analyze the data statistically. After the water solubility of a large number of compounds of various types has been determined by this method, the level of precision can be defined for various ranges.

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WATER SOLUBILITY
(GENERATOR COLUMN METHOD)

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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DISCLAIMER

Certain commercial equipment, instruments, and materials are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the Environmental Protection Agency, nor does it imply that the material, instruments, or equipment identified are necessarily the best available for the purpose.

I. INTRODUCTION

A. Background and Purpose

The water solubility of a chemical is defined as the equilibrium concentration of the chemical in a saturated aqueous solution at a given temperature and pressure. The aqueous phase solubility is an important factor in governing the movement, distribution, and rate of degradation of chemicals in the environment. Substances that are relatively water soluble are more likely to be widely distributed by the hydrologic cycle than those which are relatively insoluble. Furthermore, substances with higher water solubility are more likely to undergo microbial or chemical degradation in the environment because dissolution makes them "available" to interact and, therefore, react with other chemicals and microorganisms. Both the extent and rate of degradation via hydrolysis, photolysis, oxidation, reduction, and biodegradation depend on a chemical being soluble in water (i.e., homogeneous kinetics).

Water provides the medium in which many organisms live, and water is a major component of the internal environment of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most other chemicals are transported to

and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

The water solubility of a chemical also has an effect on its adsorption on and desorption from soils and sediments, and on volatilization from aqueous media. The more soluble a chemical substance is, the less likely it is to adsorb to soils and sediments and the less likely it is to volatilize from water. Finally, the design of most chemical tests and many ecological and health tests requires precise knowledge of the water solubility of the chemical to be tested.

B. Definitions and Units

(1) "Concentration" of a solution is the amount of solute in a given amount of solvent or solution and can be expressed as a weight/weight or weight/volume relationship. The conversion from a weight relationship to one of volume incorporates density as a factor. For dilute aqueous solutions, the density of the solvent is approximately equal to the density of the solution; thus, concentrations in mg/L are approximately equal to 10^{-3} g/ 10^3 g or parts per million (ppm); ones in $\mu\text{g/L}$ are approximately equal to 10^{-6} g/ 10^3 g or parts per billion (ppb). In addition, concentration can be expressed in terms of molarity, normality,

molality, and mole fraction. For example, to convert from weight/volume to molarity one incorporates molecular mass as a factor.

(2) "Density" is the mass of a unit volume of a material. It is a function of temperature, hence the temperature at which it is measured should be specified. For a solid, it is the density of the impermeable portion rather than the bulk density. For solids and liquids, suitable units of measurement are g/cm^3 . The density of a solution is the mass of a unit volume of the solution and suitable units of measurement are g/cm^3 .

(3) A "saturated solution" is a solution in which the dissolved solute is in equilibrium with an excess of undissolved solute; or a solution in equilibrium such that at a fixed temperature and pressure, the concentration of the solute in the solution is at its maximum value and will not change even in the presence of an excess of solute.

(4) A "solution" is a homogeneous mixture of two or more substances constituting a single phase.

(5) A "generator column" is used to produce or generate saturated solutions of a solute in a solvent. The column (see Figure 1) is packed with a solid support coated with the solute, i.e., the organic compound whose solubility is to be determined. When water (the solvent) is pumped through the column, saturated

solutions of the solute are generated. Preparation of the generator column is described under Section II.A.1.

(6) An "extractor column" is used to extract the solute from the saturated solutions produced by the generator column. After extraction onto a chromatographic support, the solute is eluted with a solvent/water mixture and subsequently analyzed by high pressure liquid chromatography (HPLC). A detailed description of the preparation of the extractor column is given in Section II.A.1.

(7) The "sample loop" is a 1/16 in. OD (1.6mm) stainless steel tube with an internal volume between 20 and 50 μ L. The loop is attached to the sample injection valve of the HPLC and is used to inject standard solutions into the mobile phase of the HPLC when determining the response factor for the recording integrator. The exact volume of the loop must be determined as described in Section II.C.1.a. when the HPLC method is used.

(8) The "response factor" (RF) is the solute concentration required to give a one unit area chromatographic peak or one unit output from the HPLC recording integrator at a particular recorder attenuation. The factor is required to convert from units of area to units of concentration. The determination of the response factor is given in Section II.C.1.b.

C. Principle of the Test Method

This test method is based on the dynamic coupled column liquid chromatographic (DCCLC) technique for determining the aqueous solubility of organic compounds that was initially developed by May et al (1978a, b), modified by DeVoe et al (1981), and finalized by Wasik et al (1981). The DCCLC technique utilizes a generator column, extractor column and HPLC coupled or interconnected to provide a continuous closed flow system. Saturated aqueous solutions of the test compound are produced by pumping water through the generator column that is packed with a solid support coated with the compound. The compound is extracted from the saturated solution onto an extractor column, then eluted from the extractor column with a solvent/water mixture and subsequently analyzed by HPLC using an ultraviolet absorption detector operating at a suitable wavelength. Chromatogram peaks are recorded and integrated using a recording integrator. The concentration of the compound in the effluent from the generator column, i.e., the water solubility of the compound, is determined from the mass of the compound (solute) extracted from a measured volume of water (solvent).

Since the HPLC method is only applicable to compounds that absorb in the ultraviolet, an alternate gas chromatographic (GC) method is used for those compounds that do not absorb in the ultraviolet. In the GC method the saturated solutions produced in

the generator column are extracted using an appropriate organic solvent that is subsequently injected into the GC for analysis of the test compound.

D. Applicability and Specificity

Procedures are described in this test guideline to determine the water solubility for liquid or solid compounds. The water solubility can be determined in very pure water, buffer solution for compounds that reversibly ionize or protonate, or in artificial seawater as a function of temperature (i.e., in the range of temperatures of environmental concern). This guideline is not applicable to the water solubility of gases.

This test guideline is designed to determine the water solubility of a solid or liquid test chemical in the range of 10 parts per billion (ppb) to 1000 ppm. For chemicals whose solubility is below 10 ppb, the water solubility should be characterized as "less than 10 ppb" with no further quantification. For solubilities greater than 1000 ppm, the shake flask method (CG-1500) should be used.

II. TEST PROCEDURE

A. Test Conditions

1. Special Laboratory Equipment

(1) Generator column - Either of two different designs are used depending on whether the eluted aqueous phase is analyzed by HPLC (Procedure A) or by solvent extraction followed by GC analysis of solvent extract (Procedure B).

The design of the generator column is shown in Figure 1. The column consists of a 6 mm (1/4-inch) O.D. Pyrex tube joined to a short enlarged section of 9 mm Pyrex tubing which in turn is connected to another section of 6 mm (1/4-inch) O.D. Pyrex tubing. Connections to the inlet Teflon tubing (1/8-inch O.D.) and to the outlet stainless steel tubing (1/16-inch O.D.) are made by means of stainless steel fittings with Teflon ferrules. The column is enclosed in a water jacket for temperature control as shown in Figure 2.

(2) Constant temperature bath with circulation pump-bath and capable of controlling temperature to $\pm 0.05^{\circ}\text{C}$. (Procedure A and B)

(3) High pressure liquid chromatograph equipped with an ultraviolet absorption detector operating at a suitable wavelength and a recording integrator. (Procedure A)

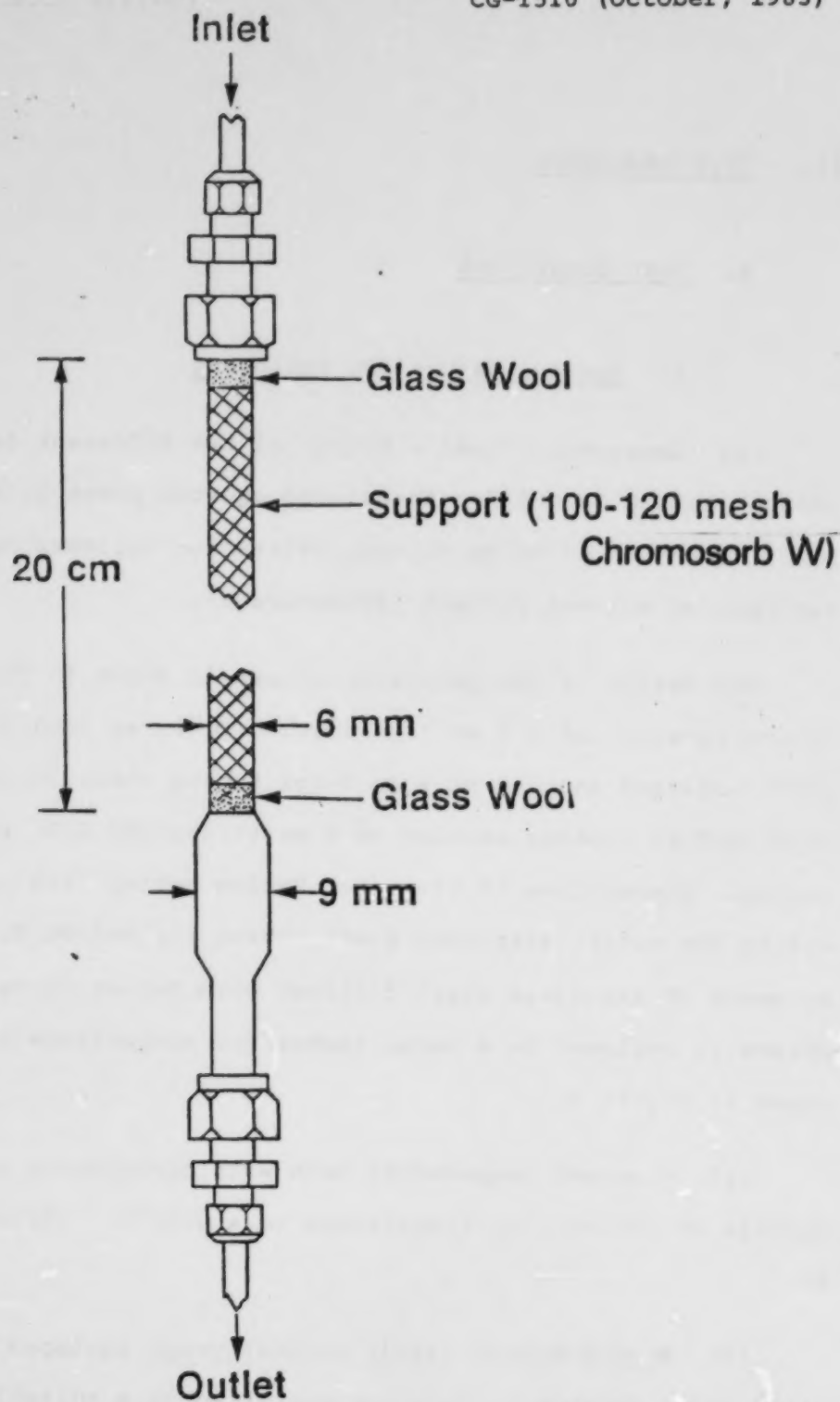


Figure 1 Generator column.

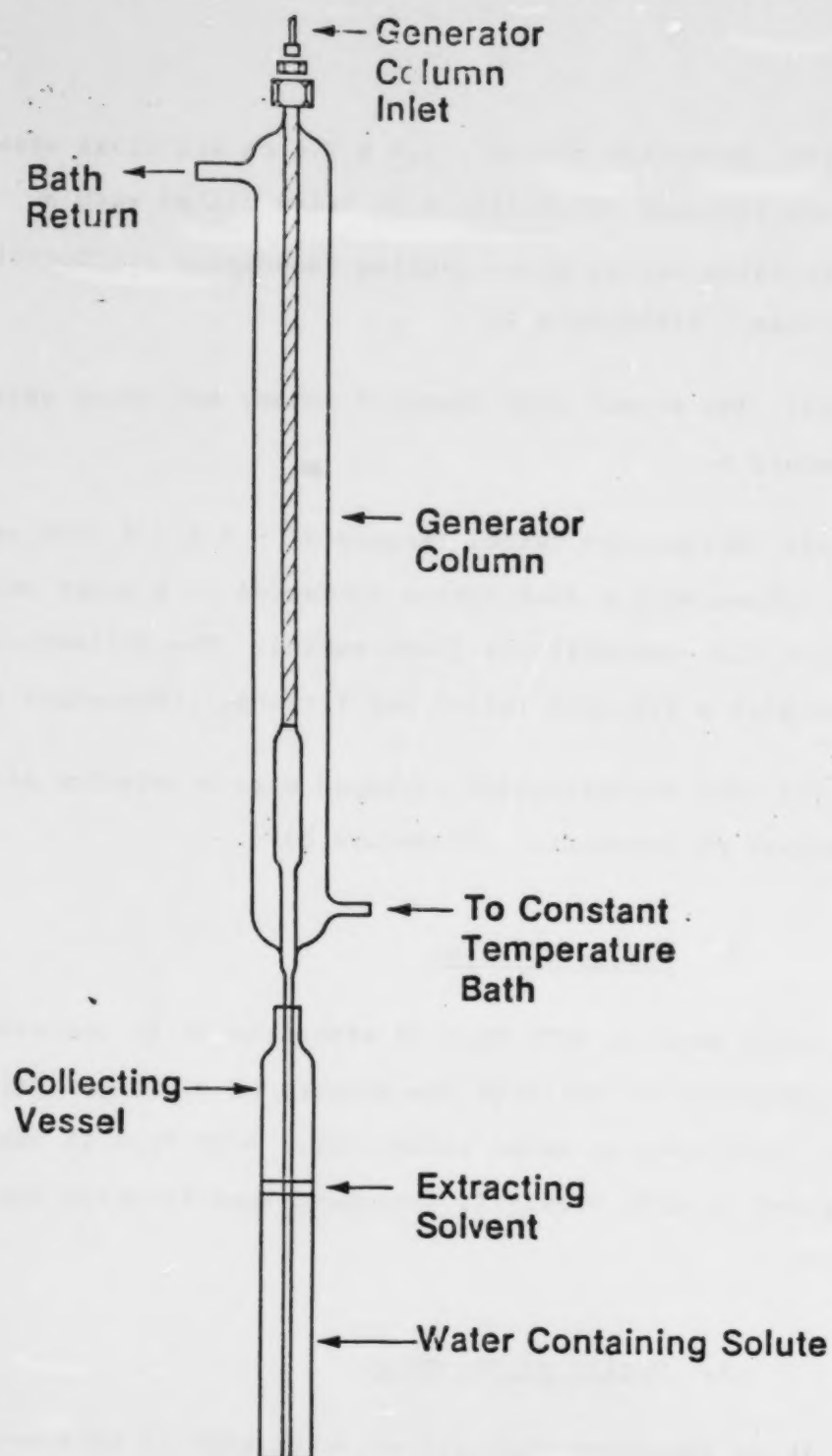


Figure 2. Setup showing generator column enclosed in a water jacket and overall arrangement of the apparatus used in the GC method.

(4) Extractor column - 6.6 X 0.6 cm stainless steel tube with end fittings containing 5 μ m frits filled with a superficially porous phase packing (Bondapack C₁₈/Corasil: Waters Associates). (Procedure A)

(5) Two 6-port high pressure rotary switching valves.
(Procedure A)

(6) Collection vessel (Figure 2) - 8 X 3/4 inch section of Pyrex tubing with a flat bottom connected to a short section of 3/8-inch O.D. borosilicate glass tubing. The collecting vessel is sealed with a 3/8-inch Teflon cap fitting. (Procedure B)

(7) Gas chromatograph equipped with a detector sensitive to the solute of interest. (Procedure B)

2. Purity of Water

Water meeting ASTM Type II standards or an equivalent grade, is recommended to minimize the effects of dissolved salts and other impurities on water solubility. ASTM Type II water is described in ASTM D1193-77, "Standard Specification for Reagent Water".

3. Purity of Solvents

It is important that all solvents used in this method be reagent or HPLC grade and contain no impurities which could interfere with the determination of the test compound.

4. Seawater

When the water solubility in seawater is desired, the artificial seawater described in Section II.B.2. should be used.

5. Effect of pH on Solubility

For chemicals that reversibly ionize or protonate (e.g., carboxylic acids, phenols, amines), experiments should be performed at pH 5.0, 7.0, and 9.0 using buffers described in Section II.B.1.

B. Preparation of Reagents and Solutions

1. Buffer Solutions

Prepare buffer solutions as follows:

pH 5.0-- To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.

pH 7.0-- To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.

pH 9.0-- To 250 mL of 0.075M borax add 69 mL of 0.1M HCl; adjust the final volume to 500 mL with reagent grade water.

Check the pH of each buffer solution with a pH meter at 25°C and adjust to pH 5.0, 7.0, or 9.0, if necessary. If the pH of the solution has changed by ± 0.2 pH units or more after the addition of the test compound, then a more concentrated buffer is required for that pH determination. The sponsor should then choose a more suitable buffer.

2. Artificial Seawater

Add the reagent-grade chemicals listed in Table 1 in the specified amounts and order to 890 mL of reagent-grade water. It is important that each chemical be dissolved before another one is added.

C. Performance of the Test

Using either Procedure A or Procedure B as described below, determine the water solubility of the test compound at 25°C in reagent grade water or buffer solution, as appropriate. Under certain circumstances, it may be necessary to determine the water solubility of a test compound at 25°C in artificial seawater. The water solubility can also be determined at other temperatures of environmental concern by adjusting the temperature of the water bath to the appropriate temperature.

Table 1--Constituents of Artificial Seawater¹

Chemical	Amount
NaF	3 mg
SrCl ₂ ·6H ₂ O	20 mg
H ₃ BO ₃	30 mg
KBr	100 mg
KCl	700 mg
CaCl ₂ ·2H ₂ O	1.47 g
Na ₂ SO ₄	4.00 g
MgCl ₂ ·6H ₂ O	10.78 g
NaCl	23.50 g
Na ₂ SiO ₃ ·9H ₂ O	20 mg
NaHCO ₃	200 mg

¹If the resulting solution is diluted to 1 cubic decimeter (1 liter), the salinity should be 34 ± 0.5 g/kg and the pH 8.0 ± 0.2 . The desired test salinity is attained by dilution at time of use.

1. Procedure A - HPLC Method

Procedure A covers the determination of the aqueous solubility of compounds which absorb in the ultraviolet. The HPLC analytical system is shown schematically in Figure 3. Two reciprocating piston pumps deliver the mobile phase (water or solvent/water mixture) through two 6-port high pressure rotary valves and a 30 X 0.6 cm C₁₈/Corosil analytical column to an ultraviolet absorption detector operating at a suitable wavelength; chromatogram peaks are recorded and integrated with a recording integrator. One of the 6-port valves is the sample injection valve used for injecting samples of standard solutions of the solute in an appropriate concentration for determining response factors or standard solutions of basic chromate for determining the sample loop volume. The other 6-port valve in the system serves as a switching valve for the extractor column which is used to remove solute from the aqueous solutions.

The general procedure for analyzing the aqueous phase is as follows; a detailed procedure is given in part d. of this section: 1) Direct the aqueous solution to "Waste" (see Figure 3) with the switching valve in the inject position in order to equilibrate internal surfaces with the solution, thus insuring that the analyzed sample would not be depleted by solute adsorption on surfaces upstream from the valve. 2) At the same time, water is pumped from the HPLC pumps in order to displace the solvent from the extractor column. 3) The switching valve is

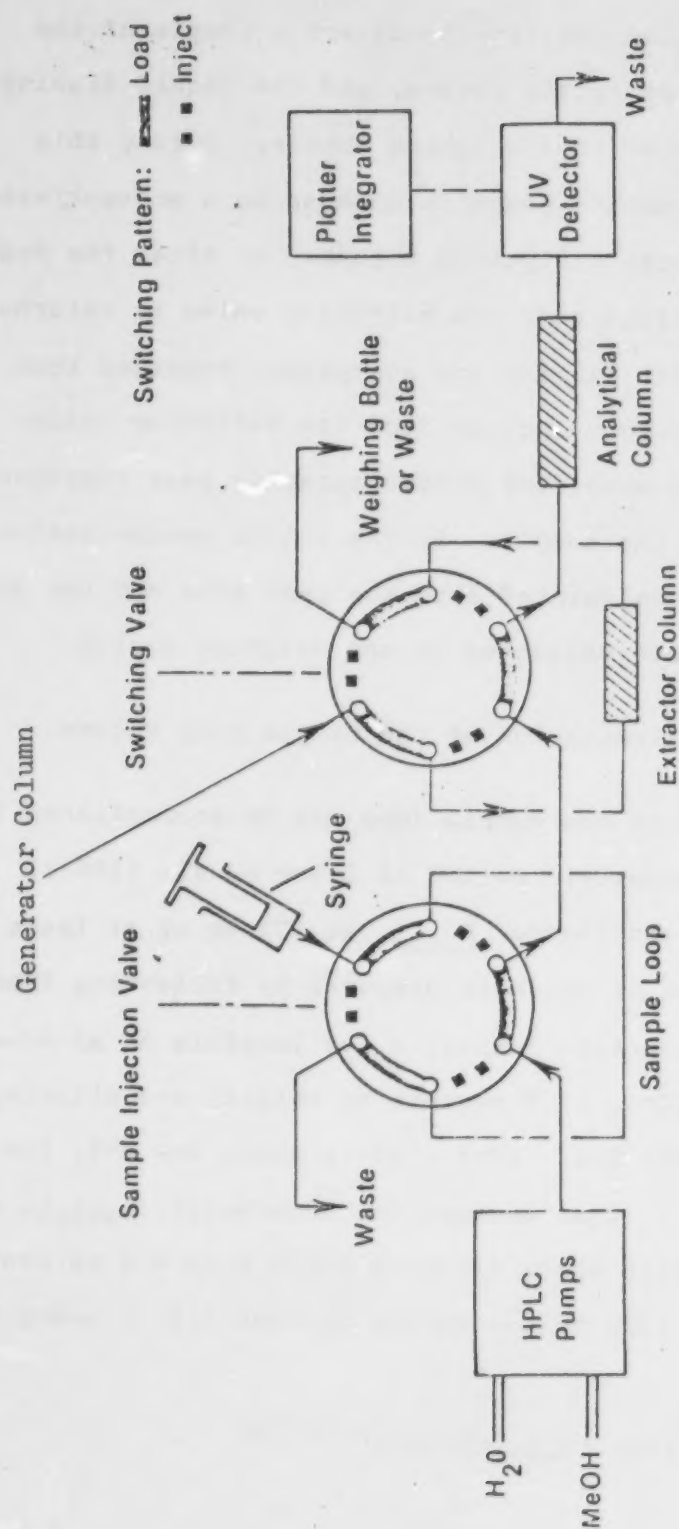


Figure 3 Schematic of HPLC - generator column flow system.

next changed to the load position to divert a sample of the solution through the extractor column, and the liquid leaving this column is collected in a weighing bottle. During this extraction step, the mobile phase is changed to a solvent/water mixture to condition the analytical column. 4) After the desired volume of sample is extracted, the switching valve is returned to the inject position for elution and analysis. Provided that there is no breakthrough of solute from the extractor column during the extraction step, the chromatographic peak represents all of the solute in the sample. 5) The solute concentration in the aqueous phase is calculated from the peak area and the weight of the extracted liquid collected in the weighing bottle.

a. Determination of the Sample Loop Volume

Accurate measurement of the sample loop may be accomplished by using the spectrophotometric method of Devoe et al. (1981). For this method measure absorbance, A_{loop} , at 373 nm of at least three solutions, each of which is prepared by collecting from the sample valve an appropriate number, n , of loopfuls of an aqueous stock solution of K_2CrO_4 (1.3 percent by weight) and diluting to 50 mL with 0.2 percent KOH. (For a 20- L loop, use $n=5$; for a 50- L loop, use $n=2$.) Also measure the absorbance, A_{stock} , of the same stock solution after diluting 1:500 with 0.2 percent KOH. Calculate the loop volume to the nearest 0.1 L using the relation:

$$V_{loop} = (A_{loop}/A_{stock})(10^{-4}/n)$$

b. Determination of the Response Factor (RF)

For all determinations adjust the mobile phase solvent/water ratio and flow rate to obtain a reasonable retention time on the HPLC column. For example, typical concentrations of solvent in the mobile phase range from 50 to 100 percent while flow rates range from 1 to 3 mL/min; these conditions give a 3 to 5 minute retention time.

Prepare standard solutions of known concentrations of the solute in a suitable solvent. Concentrations must give a recorder response within the maximum response of the detector. Inject samples of each standard solution into the HPLC system using the calibrated sample loop. Obtain an average peak area from at least three injections of each standard sample at a set absorbance unit full scale (AUFS), i.e., at the same absorbance scale attenuation setting.

Calculate the response factor from the following equation:

$$\text{Response Factor (RF)} = \frac{\text{Concentration (M)}}{(\text{Average Area})(\text{AUFS})}$$

c. Loading of the Generator Column

The design of the generator column was described in Section II.A.1 and is shown in Figure 1. To pack the column, a plug of silanized glass wool is inserted into one end of the 6mm Pyrex

tubing. Silanized diatomaceous silica support (about 0.5g 100-120 mesh Chromosorb W chromatographic support material) is poured into the tube with tapping and retained with a second plug of silanized glass wool.

If the solute is a liquid, the column is loaded by pulling the liquid solute through the dry support with gentle suction. If the solute is a solid, a 1% solution of the solid in a volatile solvent is added to the dry packing. The solvent is then distilled off the column under reduced pressure. After loading the column draw water up through the column to remove entrapped air.

d. Analysis of the Solute

Use the following procedure to collect and analyze the solute.

1. Pump water to the generator column by means of a minipump or pressurized water reservoir as shown in Figure 4. With the switching valve (Figure 3) in the inject position (i.e., water to waste), pump water through the generator column at a flow rate of approximately 1 mL/min for approximately 5 minutes to bring the system into equilibrium.
2. Flush out the solvent that remains in the system from previous runs by changing the mobile phase to 100% H₂O and allowing the water to reach the HPLC

detector, as indicated by a negative reading. As soon as this occurs, place a 25 mL weighing bottle (weighed to the nearest mg) at the waste position and immediately turn the switching valve to the load position.

3. Collect an amount of water (as determined by trial and error) in the weighing bottle, corresponding to the amount of solute adsorbed by the extractor column that gives a large on-scale detector response. During this extraction step, switch back to the original HPLC mobile phase composition, i.e., solvent/water mixture, to condition the HPLC analytical column.
4. After the desired volume of sample has been extracted, turn the switching valve back to the inject position (Figure 3); at the same time turn on the recording integrator. The solvent/water mobile phase will elute the solute from the extractor column and transfer the solute to the HPLC analytical column.
5. Remove the weighing bottle, cap it and replace it with the waste container. Determine the weight of water collected to the nearest mg and record the corresponding peak area. Using the same AUFS setting repeat the analysis of the solute at least

two more times and determine the average ratio of peak area to grams of water collected. Calculate the solute solubility in water using the following equation:

$$s = (997 \text{ g/L})(\text{RF})(V_{\text{loop}})(\text{AUFS})(\text{R})$$

where s = solubility (M)

RF = response factor

V_{loop} = sample loop volume (L)

R = ratio of area to grams of water.

2. Procedure B - GC Method

In the GC method, aqueous solutions from the generator column enter a collecting vessel (Figure 2) containing a known weight of extracting solvent which is immiscible in water. The outlet of the generator column is positioned such that the aqueous phase always enters below the extracting solvent. After the aqueous phase is collected, the collecting vessel is stoppered and the quantity of aqueous phase is determined by weighing. The solvent and the aqueous phase are equilibrated by slowly rotating the collecting vessel. A small amount of the extracting solvent is then removed and injected into a gas chromatograph equipped with an appropriate detector. The solute concentration in the aqueous phase is determined from a calibration curve constructed using known concentrations of the solute.

a. Determination of Calibration Curve

Prepare solute standard solutions of concentrations covering the range of the solute solubility. Select a column and optimum GC operating conditions for resolution between the solute and solvent and the solute and extracting solvent. Inject a known volume of each standard solution into the injection port of the GC. For each standard solution determine the average of the ratio R of peak area to volume (in μL) for three (3) chromatographic peaks from three (3) injections.

After running all the standard solutions, determine the coefficients, a and b, using a linear regression equation of concentration (c) vs. R in the following form

$$c = aR + b.$$

b. Loading of the Generator Column

The generator column is packed and loaded with solute in the same manner as for the HPLC method (see Section II.C.1.c.). As shown in Figure 2, attach approximately 20 cm of straight stainless steel tubing to the bottom of the generator column. Connect the top of the generator column to a water reservoir (Figure 4) using Teflon tubing. Use air or nitrogen pressure (5 PSI) from an air or nitrogen cylinder to force water from the reservoir through the column. Collect water in an Erlenmeyer

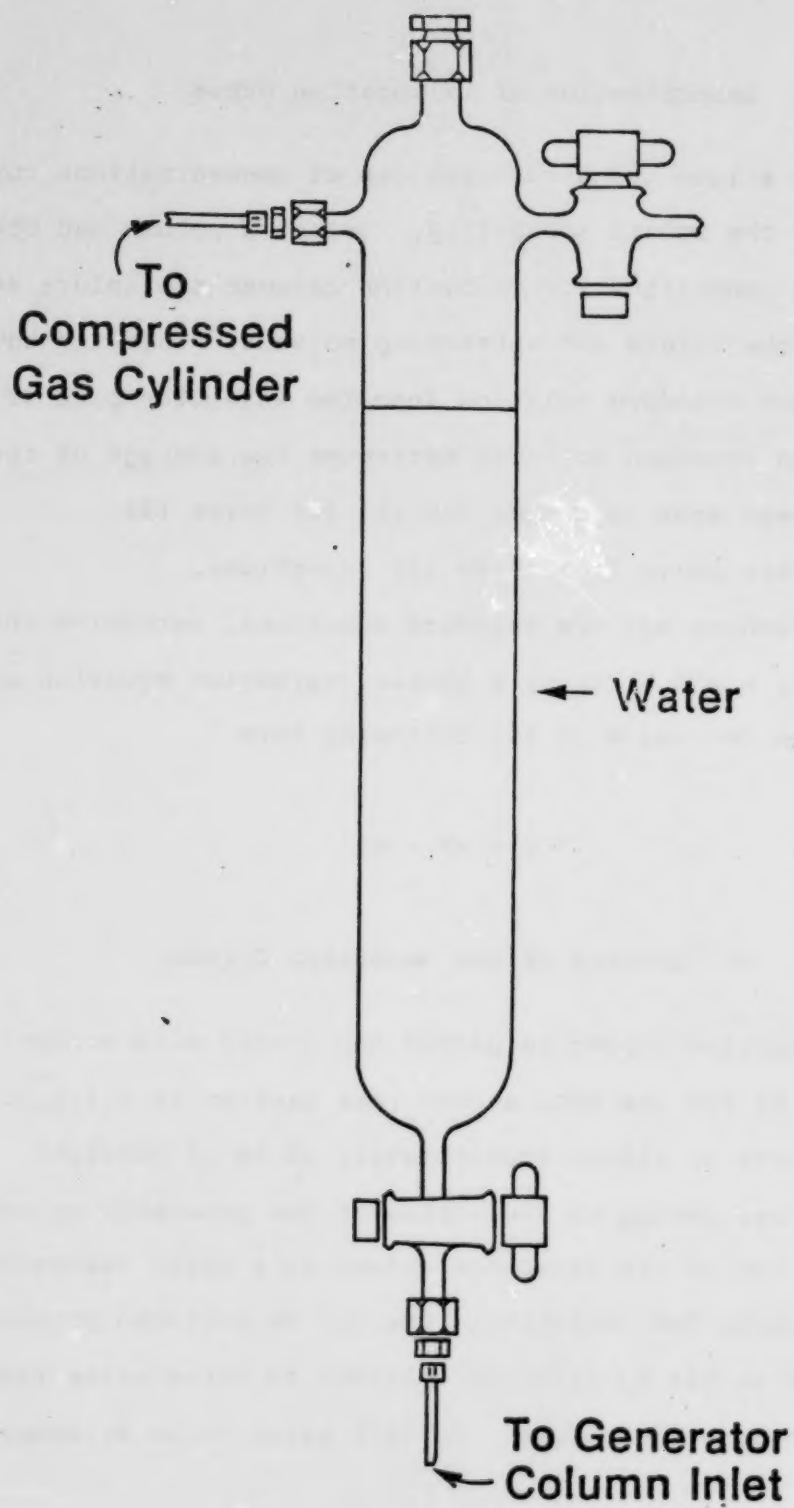


Figure 4 Water reservoir for GC method.

flask for approximately 15 minutes while the solute concentration in water equilibrates; longer time may be required for less soluble compounds.

c. Collection and Extraction of the Solute

During the equilibration time, add a known weight of extracting solvent to a collection vessel which can be capped. The extracting solvent should cover the bottom of the collection vessel to a depth sufficient to submerge the collecting tube but still maintain 100:1 water/solvent ratio. Record the weight (to the nearest mg) of a collection vessel with cap and extracting solvent. Place the collection vessel under the generator column so that water from the collecting tube enters below the level of the extracting solvent (Figure 2). When the collection vessel is filled, remove it from under the generator column, replace cap, and weigh the filled vessel. Determine the weight of water collected. Before analyzing for the solute, gently shake the collection vessel contents for approximately 30 min., controlling the rate of shaking so as not to form an emulsion; rotating the flask end over end five times per minute is sufficient.

d. Analysis of the Solute

After shaking, allow the collection vessel to stand for approximately 30 minutes; then remove a known volume of the extracting solvent from the vessel using a microliter syringe and

inject it into the G.C. Record the ratio of peak area to volume injected and, from the regression equation of the calibration line, determine the concentration of solute in the extracting solvent. The concentration of solute in water $C(M)$ is determined from the following equation

$$C(M) = (C_{es}) \frac{d_{H_2O}}{d_{es}} \frac{g_{es}}{g_{H_2O}}$$

where C_{es} is the concentration of solute in extracting solvent (M), d_{H_2O} and d_{es} are the densities of water and extracting solvent, respectively, and g_{es} and g_{H_2O} are the grams of extracting solvent and water, respectively, contained in the collection vessel.

Make replicate injections from each collecting vessel to determine the average solute concentration in water for each vessel. To make sure the generator column has reached equilibrium, run at least two additional (for a total of three) collection vessels and analyze the extracted solute as described above. Calculate the water solubility of the solute from the average solute concentration in the three vessels.

3. Modification of Procedures for Potential Problems

a. Decomposition of the Test Compound

If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be

obtained, then it will be necessary to carry out detailed transformation studies; e.g., hydrolysis (Test Guideline CG-5000). If decomposition is due to aqueous photolysis, then it will be necessary to carry out water solubility studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

III. DATA AND REPORTING

A. Test Report

For each set of conditions, (e.g., temperature, pure water, buffer solution, artificial seawater) required for the study, provide the water solubility value for each of three determinations, the mean value, and the standard deviation.

For compounds that decompose at a rate such that a precise value for the water solubility cannot be obtained, provide a statement to that effect.

For compounds with water solubility below 10 ppb, report the value as "less than 10 ppb".

B. Specific Analytical, Calibration and Recovery Procedures

For the HPLC method describe and/or report:

(1) The method used to determine the sample loop volume and the average and standard deviation of that volume.

(2) The average and standard deviation of the response factor.

(3) Any changes made or problems encountered in the test procedure.

For the GC method report:

(1) The column and GC operating conditions of temperature and flow rate.

(2) The average and standard deviation of the average area/ μ L obtained for each of the standard solutions.

(3) The form of the regression equation obtained in the calibration procedure.

(4) The extracting solvent used.

(5) The average and standard deviation of solute concentration in each collection vessel.

(6) Any changes made or problems encountered in the test procedure.

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October, 1983

- WATER SOLUBILITY
(GENERATOR COLUMN METHOD)

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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I. NEED FOR THE TEST

The water solubility of a compound can be defined as the equilibrium concentration of the compound in a saturated aqueous solution at a given temperature and pressure. The water solubility is a fundamental physical property of a compound. It is an essential characteristic for determining that compound's movement and distribution in the environment and, therefore, its potential effects on living organisms. Highly soluble compounds are more likely to be distributed by the hydrologic cycle than less soluble compounds. The degree of water solubility of a compound can affect its adsorption and desorption on soils and sediments and ease of volatility from aquatic systems. Substances that are more soluble in water are less likely to adsorb to soils and sediments and less likely to volatilize from water. Transformations such as hydrolysis, photolysis, oxidation, reduction, and biodegradation in water proceed more rapidly when the compound is dissolved in water (i.e., homogeneous kinetics). The potential importance of these transformations is, therefore, a function of the ability of a compound to dissolve in water. Finally, the design of most chemical tests and many ecological and health tests requires accurate knowledge of the water solubility of the compound to be tested.

Virtually all modeling systems devised to determine the distribution of a compound in the environment require water solubility data.

Although it is generally agreed that the actual physical measurement of water solubility is the most accurate and desirable approach (Mader and Grady 1970), because of the lack of data and poor analytical methodology, many attempts have been made to correlate water solubility with other physical parameters such as structure, enthalpy of fusion, melting point, molar volume, and the octanol/water partition coefficient. The regression equations from these correlations enable the water solubility of a wide variety of compounds to be estimated. The most important of these correlations is between the water solubility and the octanol/water partition coefficient (K_{ow}) (Chiou et al. 1977, Yalkowsky and Valvani 1979, 1980, 1981), which enables a reasonable estimate of a compound's water solubility to be made from its K_{ow} . [Of course these regression equations are equally applicable to estimating K_{ow} from water solubility (see CG/CS-1400)].

Water provides the medium in which many organisms live, and water is a major component of the internal portion of all living organisms (except for dormant stages of certain life forms). Even organisms that are adapted to life in a gaseous environment require water for normal functioning. Water is the medium through which most chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in

water will be a major determinant for movement through the environment and entry into living systems.

Water solubility is an essential parameter for assessing the environmental partitioning of all solid or liquid chemicals. However, water solubility is usually not useful for gases because their solubility in water is measured when the gas above the water is at a partial pressure of one atmosphere which is several orders of magnitude greater than those existing under environmental conditions. Thus, water solubility of gases does not generally apply to environmental assessment because the actual partial pressure of a gas in the environment is extremely low.

II. SCIENTIFIC ASPECTS

A. Rationale for the Selection of the Test Method

Analytical methods for the determination of water solubility consist of obtaining an equilibrium saturated aqueous solution of the compound and analyzing the solution by some suitable physical or chemical method. Equilibrium may be obtained by intimately mixing the solute and solvent. Separation of the phases can then be accomplished by filtration or decantation, usually followed by centrifugation. The supernatant is then analyzed by a compound-specific analytical technique.

The solubility of a compound in water at equilibrium is a function of temperature, pressure, and purity of solute and

water. A major practical difficulty is determining that equilibrium has actually been attained. Mader and Grady (1970) have described two general methods to determine the attainment of equilibrium. The concentration vs. time method involves periodic sampling over meaningful periods of time; when the concentration has reached a plateau, equilibrium has been attained. The undersaturation/oversaturation method refers to approaching equilibrium from both possible non-equilibrium conditions. One of two identical solutions is heated to a temperature well above the required test temperature so that the solubility is increased. Then both solutions are placed in a thermostatic bath to equilibrate at the required test temperature. The heated solution becomes supersaturated (oversaturated) when its temperature decreases and reaches equilibrium as the concentration of the dissolved solute decreases to a plateau at the required test temperature. The other solution is undersaturated and reaches equilibrium as the concentration of dissolved solute increases to a plateau at the required test temperature. If identical concentrations in aliquots of these two samples are obtained after a period of time, equilibrium has been reached. Research sponsored by EPA at the Battelle Institute demonstrated that the under/oversaturation method will give poor analytical results if the compound tested undergoes a phase transition in the temperature range utilized (EPA 1981). Thus, a low melting solid may recrystallize at a very slow rate after being heated to elevated temperatures and make separation of excess solute from the aqueous phase extremely difficult.

Other potential problems include accelerated decomposition of the test material at elevated temperatures and dramatically different changes in solubility rates of impurities vs. test compound with change in temperature.

The methods described in the Test Guideline CG-1510 are based on the dynamic coupled column liquid chromatographic (DCCLC) technique that was initially developed by May et al. (1978a,b), then modified by DeVoe et al. (1981), and finalized by Wasik et al. (1981). The techniques described herein utilize a generator column, extractor column or vessel and a high pressure liquid chromatograph (HPLC) or gas chromatograph (GC). In the HPLC method saturated aqueous solutions of the test compound are produced by pumping water through a generator column packed with a solid support coated with the test compound. Subsequently, the test compound is quantitatively extracted from the saturated solution onto an extractor column containing a superficially porous reverse phase packing. After extraction, the compound is eluted from the extractor column with a solvent/water mixture and analyzed by HPLC using an ultraviolet absorption detector operating at a wavelength appropriate to detect the compound.

Since the HPLC method is only applicable to compounds that absorb in the ultraviolet, an alternate gas chromatographic (GC) method is used for those compounds that do not absorb in the ultraviolet. In the GC method, the saturated solutions produced in the generator column are extracted using an appropriate organic solvent that is subsequently injected into the GC for analysis of the test compound.

Both of these methods have several advantages over the standard shake flask methods. When determining the water solubility of hydrophobic compounds, the concentration of solute (the compound) in the aqueous phase is very low. In the shake flask method, significant errors in this concentration can occur through the formation of colloidal dispersions or emulsions during shaking, adsorption of the solute to surfaces of transfer vessels and loss of a volatile solute to the atmosphere. Although these errors can be reduced in each case by centrifugation, prerinsing of all transfer vessels, avoiding exposure of the aqueous phase to the atmosphere and allowing sufficient time for equilibration, these additional steps are inconvenient, time consuming, and increase the cost of analysis.

All of these problems are avoided in both the HPLC and GC methods where the use of the generator column to produce saturated solutions avoids the shaking that produces emulsions. Errors due to volatilization are eliminated in the HPLC method because the generator column, extractor columns and HPLC are coupled together in a closed system where there is no exposure to the atmosphere. In the GC method, the sampling technique prevents the saturated solutions from being exposed to the atmosphere. Both procedures use a chromatographic column, where equilibrium is attained rapidly, for the generation of a saturated solution. In addition, both methods minimize adsorption by presaturating the walls of the transfer lines with the test compound during the column conditioning process and by rapid (< 75 milliseconds) transfer of the saturated solution to the extractor column.

Using the generator column methods, aqueous solubilities have been systematically determined for seven different classes of chemicals covering a wide range in solubility (5 ppb to 1000 ppm). The results are in good agreement with experimental literature data obtained by the conventional shake-flask method (Wasik et al. 1981). In addition, the method has been used to determine the water solubility of polynuclear aromatic hydrocarbons whose solubilities were as low as 1.8 ppb for chrysene at 25°C (May and Wasik, 1978a, b).

B. Other Methods for Determining Water Solubility

Both interferometry (Adams 1915, Mader and Grady 1970) and nephelometry (Davis and Parke 1942) have been used in determining the water solubility of sparingly soluble organic compounds. However, these methods do not have general applicability and are beset with other limitations. These methods are discussed in detail in the support document for the shake flask method (CS-1500).

The shake flask method (Test Guideline CG-1500) should be used for compounds whose water solubility exceeds 1000 ppm (0.1 percent). The generator column method is most suitable for compounds that have a water solubility lower than 1000 ppm for several reasons: 1) the solid support used in the generator column has a finite surface area and, therefore, can only hold a limited amount of solute. For more soluble compounds this amount of solute would be inadequate to saturate a sufficient amount of

be inadequate to saturate a sufficient amount of water for replicate analyses; 2) for more soluble compounds the concentration of solute in solution would exceed the capacity of the extractor column and cause "break through", thereby resulting in erroneous results; and 3) the amount of water collected in the extraction step must be sufficient to quantify. For compounds more soluble than 1000 ppm, the amount of water collected is insufficient to obtain reproducible results.

C. Rationale for the Selection of the
Test Conditions

1. Temperature

The equilibrium water solubility of a substance is a constant at a fixed temperature and pressure. For most solid and some liquid compounds, an increase in temperature generally results in an increase in water solubility, particularly above 20°C. The rate of change in water solubility with temperature is not linear for a given chemical and varies dramatically from one substance to another (Mader and Grady 1970). For many solid compounds, the following relationship describes concentration (water solubility) as a function of temperature:

$$\ln \frac{1}{X_a} = \frac{\Delta H}{R} \left(\frac{1}{T} - \frac{1}{T_f} \right),$$

where X_a is the mole fraction of compound a, ΔH is the enthalpy of fusion, T_f is the thermodynamic temperature of fusion, and R

is the universal gas constant (Bigger and Riggs 1974). The equation can be put into the form $\log_{10} C = -\frac{A}{T} + B$, where C = molar concentration, T is the thermodynamic temperature and A and B are constants. By determining C as a function of T (e.g., at 5, 15, and 25°C), the data can be used to determine A and B (i.e., plot $\log_{10} C$ vs. $1/T$; the slope of the line is A and the intercept is B). Thus, one can use this equation to estimate C at any environmental temperature of concern. As a working estimate for ambient temperatures, one can generally expect a doubling of the solubility of solids with an increase of 10°C.

Since water solubility values will change with temperature (Mader and Grady 1970), the temperature should be controlled. For the generator column methods, which are very precise, the temperature should be controlled to ± 0.05 °C. This is a condition easily achieved by standard temperature control devices for water baths. The temperature chosen for this test guideline is 25°C since this is the temperature traditionally used for reporting physical properties. Solubility determinations may be requested at additional temperatures of environmental concern for specific compounds or classes of compounds.

2. Purity of Water

Dissolved salts and other impurities can affect the water solubility of a compound, necessitating the use of very pure water (Mader and Grady 1970). ASTM Type II reagent grade water (ASTM 1978), or an equivalent grade, is recommended for use in

this test guideline. The use of bacteria-free water, to prevent biodegradation of the test compound during the test, is not required because the short duration of the test minimize any losses due to biodegradation.

3. Purity of Solvents

Organic solvents that come into contact with the test material and aqueous solution must be as pure as practicable. Trace organic impurities that are soluble in water can alter the water solubility of the test material as well as interfere with the analysis of the concentration of the test material.

4. Seawater

Organic compounds are often released or transported to seawater. The presence of dissolved salts will usually alter the true water solubility of a compound due to what is called the salting out effect. Typically, the water solubility of an organic compound will be less in seawater than in pure water at a fixed temperature (Long and McDevit 1952). Data on solubility in seawater are needed to design ecological testing of marine species and for modeling marine environments. Salinity varies in different marine environments. In order to determine solubility under uniform conditions, a formulation for artificial seawater is specified in the test guideline (Kester et al. 1967, EPA 1975a).

5. Effect of pH on Solubility

It is known that the water solubility of some organic compounds will change with a change in the pH of the solution (Cheung and Biggar 1974). This change will not be dramatic unless the compound contains readily ionizable or protonated groups. For carboxylic acids, amines, and compounds which reversibly ionize or protonate in water, it is necessary to measure the water solubility at pH values of 5.0, 7.0, and 9.0. These pH values are representative of those found in natural aquatic ecosystems, groundwaters, and rainwaters.

The specified buffer solutions have a capacity to neutralize approximately 100-500 ppm of dissolved test compound (depending on its molecular weight) without a significant change in pH and, therefore, will be satisfactory for most test compounds. A change in pH of 0.2 units signifies inadequate buffer capacity. In such cases, the choice of buffer solution should be made by the sponsor.

D. Test Data Required

The HPLC and GC analytical techniques used in this test method allow the determination of the concentration of organic compounds in water as low as the parts per trillion range with an accuracy of ± 3 percent. However, at solubilities below 10 ppb, organic substances are so hydrophobic that they do not dissolve, but disperse in water, forming micelles. Thus, for substances whose water solubility is below 10 ppb, the test

guideline suggests that the water solubility be characterized as "less than 10 ppb" with no further quantification.

The test guideline requires that data be reported not only on the mean and standard deviation of the water solubility of the test compound, but also on the calibration of the sample loop volume and response factor for the HPLC method. For the GC method, data on the calibration procedure using the standard solutions, the resulting regression equation, and results from replicate GC runs should be reported. The reporting of these data is required because they are essential in calculating the water solubility of the test compound.

E. Statistical Analysis of the Data

The precision of the water solubility as determined by the proposed test method has not yet been established. However, as the solubility decreases, the precision is expected to become poorer. The precision is also a function of the nature of the specific compound and the analytical procedure used. Reliable precision can be stated only for various solubility ranges after making numerous replicate determinations. However, in order to minimize costs, only three determinations are used to calculate the mean water solubility and its standard deviation. As a matter of standard laboratory practice, the mean and standard deviation are required from three runs of each calibration and ancillary procedures.

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SOIL THIN-LAYER CHROMATOGRAPHY

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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SOIL THIN-LAYER CHROMATOGRAPHYI. INTRODUCTIONA. Background and Purpose

Leaching of chemicals through soil is an important process which affects a chemical's distribution in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Knowledge of the leaching potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment.

Chemical leaching also affects the assessment of ecological and human health effects of chemicals. If a chemical reaches ground water, deleterious human health effects may arise due to the consumption of drinking water. If a chemical remains at the soil surface, deleterious environmental and human health effects may arise due to an increased concentration of the chemical in the zone of plant growth, possibly resulting in contamination of human food supplies.

Soil thin layer chromatography (TLC) is a qualitative screening tool suitable for obtaining an estimate of a chemical's leaching potential. This test is one of several tests which can be used in obtaining a rough estimation of a chemical's leaching potential.

B. Definitions and Units

"Cation exchange capacity" (CEC) is the sum total of exchangeable cations that a soil can adsorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meq/100g) or milliequivalents of negative charge per gram (meq/g) of soil.

"Particle size analysis" is the determination of the various amounts of the different particle sizes in a soil sample (i.e., sand, silt, clay) usually by sedimentation, sieving, micrometry or combinations of these methods. The names and size limits of these particles as widely used in the United States are:

very coarse sand	2.0 to 1.0 mm dia.
coarse sand	1.0 to 0.5 mm
medium sand	0.5 to 0.25 mm
fine sand	0.25 to 0.125 mm
very fine sand	0.125 to 0.062 mm
silt	0.062 to 0.002 mm
clay	<0.002 mm

" R_f " is the furthest distance traveled by a test material on a thin-layer chromatography plate divided by the distance traveled by a solvent front (arbitrarily set at 10.0 cm in soil TLC studies).

"Soil" is the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants; its formation and properties are determined by various factors such as parent material, climate, macro- and microorganisms, topography, and time.

"Soil aggregate" is the combination or arrangement of soil separates (sand, silt, clay) into secondary units. These units may be arranged in the profile in a distinctive characteristic pattern that can be classified on the basis of size, shape, and degree of distinctness into classes, type, and grades.

"Soil classification" is the systematic arrangement of soils into groups or categories. Broad groupings are made on the basis of general characteristics, subdivisions, on the basis of more detailed differences in specific properties. The soil classification system used today in the United States is the 7th Approximation Comprehensive System. The ranking of subdivisions under the system is: order, suborder, greatgroup, family and series.

"Soil horizon" is a layer of soil approximately parallel to the land surface. Adjacent layers differ in physical, chemical, and biological properties or characteristics such as color, structure, texture, consistency, kinds, and numbers of organisms present, and degree of acidity or alkalinity.

"Soil order" is the broadest category of soil classification and is based on general similarities of physical/chemical properties. The formation by similar genetic processes causes these similarities. The soil orders found in the United States are: Alfisol, Aridisol, Entisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol, and Vertisol.

"Soil organic matter" is the organic fraction of the soil; it includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by the microbial population.

"Soil pH" is the negative logarithm to the base 10 of the hydrogen ion activity of a soil as determined by means of a suitable sensing electrode coupled with a suitable reference electrode at a 1:1 soil:water ratio.

"Soil series" is the basic unit of soil classification and is a subdivision of a family. A series consists of soils that were developed under comparable climatic and vegetational conditions. The soils comprising a series are essentially alike in all major profile characteristics except for the texture of the "A" horizon (i.e. the surface layer of soil).

"Soil texture" refers to the classification of soils based on the relative proportions of the various soil separates present. The soil textural classes are: clay, sandy clay, silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand.

C. Principle of the Test Method

Before 1968, methods of investigating the mobility of nonvolatile organic chemicals within soils were based on the use of field analysis, soil adsorption isotherms, and soil columns. In 1968, Helling and Turner introduced soil thin layer chromatography (soil TLC) as an alternative procedure; it is analogous to conventional TLC, with the use of soil instead of silica gels, oxides, etc., as the adsorbent phase.

The papers by Helling (1968, 1971a, 1971b, 1971c) and Helling and Turner (1968) were the basis of this test guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiates the experimental conditions specified in the guideline.

The soil TLC offers many desirable features. First, mobility results are reproducible. Mass transfer and diffusion components are distinguishable. The method has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It yields data that are amenable to statistical analyses. A chemical extraction-mass balance procedure to elicit information on degradation and chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease with which the R_f and mass balance are performed will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques for measuring the chemical.

D. Applicability and Specificity

Soil TLC can be used to determine the soil mobility of sparingly water soluble to infinitely soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested since the literature indicates that these chemicals are, in general, immobile (Goring and Hamaker, 1972). However this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

Soil TLC may be used to test the mobility of volatile chemicals by placing a clean plate over the spotted soil TLC plate and then placing both plates in a closed chromatographic chamber.

Soil TLC was originally designed for use with soils. The literature shows no published use of this method with sediments as the absorbent phase, probably due to the fact that sediment surface properties change significantly during air drying. It is extremely important that the TLC plate with the adsorbent be air dried before leaching studies can be undertaken.

II. TEST PROCEDURES

A. Test Conditions

- (1) Equipment required: Distilled-deionized water adjusted to pH 7 by boiling to remove CO₂; clean glass plates (TLC); glass rods or a variable thickness plate spreader; masking tape; closed chromatographic chambers; analytical instrumentation necessary and appropriate for the detection and quantitative analysis of the test chemical;
- (2) the test procedure may be run at $23 \pm 5^{\circ}\text{C}$; and
- (3) it is recommended that three replicate plates for each soil be used.

B. Test Procedures

- (1) To reduce aggregate size before or during sieving, crush and grind the air dried soil very, very gently;
- (2) sieve air dried soils with a 250 micrometer sieve;
- (3) add water to the sieved soil until a smooth, moderately fluid slurry is attained (approximately 3/4 ml H₂O added for each gram of soil);

- (4) spread the slurry evenly and quickly across the clean glass plate using a variable thickness plate spreader, a glass rod, or other available method. If a glass rod is used, control the layer thickness by affixing multiple layers of masking tape along the plate edges. Soil layer thickness should be 0.50 - 0.75 mm;
- (5) air dry the plates at 25°C for a minimum of 24 hours after uniform slurry application is achieved;
- (6) scribe a horizontal line 11.5 cm above the base through the soil layer down to the glass so as to stop solvent movement;
- (7) spot the test chemical, in solution, 1.5 cm above the base. For radiolabeled materials, 0.5 - 5 μ g containing 0.01 - 0.03 μ Ci of ^{14}C labeled compound may be used;
- (8) if the compound is volatile, it is extremely important that a clean plate be placed over the soil TLC plate to impede volatilization;
- (9) immerse the plate with the base down at some angle from the vertical in a closed chromatographic chamber containing H_2O at a height of 0.5 cm;
- (10) allow the solvent front to migrate to the 11.5 cm line before removing the plates from the chamber;
- (11) determine the R_f values. Zonal extraction, plate scanning, or any other method or combination of methods suitable for detection of the parent test chemical may be used; and

- (12) determine the amount of the parent test chemical on the entire soil TLC plate after test chemical migration. Any method or combination of methods suitable for the extraction and quantitative detection of the parent test chemical may be used.

III. DATA AND REPORTING

Report the following information.

- (1) Temperature at which the test was conducted;
- (2) amount of the test chemical applied and amount recovered from the plates;
- (3) detailed description of the analytical technique used in the R_f determination, the chemical extraction, and the quantitative recovery and analysis of the parent chemical;
- (4) the mean frontal R_f value with the standard deviation for each soil tested;
- (5) a photograph or diagram of the TLC plate which shows the entire leaching pattern (from 1.5 to 11.5 cm);
- (6) soil information: soil order, series, texture, sampling location, horizon, general clay fraction mineralogy; and
- (7) soil physical/chemical properties: percent sand, percent silt and percent clay (particle size analysis); percent organic matter; pH (soil to water ratio, 1:1); and cation exchange capacity.

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SOIL THIN LAYER CHROMATOGRAPHY

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WASHINGTON, DC 20460

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SOIL THIN LAYER CHROMATOGRAPHYI. NEED FOR THE TEST

Leaching of chemicals through soil is an important process which affects a chemical's distribution in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Knowledge of the leaching potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment.

Chemical leaching also affects the assessment of ecological and human health effects of chemicals. If a chemical reaches ground water, deleterious human health effects may arise due to the consumption of drinking water. If a chemical remains at the soil surface, deleterious environmental and human health effects may arise due to an increased concentration of the chemical in the zone of plant growth, possibly resulting in contamination of human food supplies.

Soil thin layer chromatography (TLC) is a qualitative screening tool suitable for obtaining an estimate of a chemical's leaching potential. This test is one of several tests which can be used in obtaining a rough estimation of a chemical's leaching potential.

II. SCIENTIFIC ASPECTS OF SOIL LEACHING

A. Introduction

Since chemical leaching in soils is affected by a large number of interacting processes (Hamaker 1975) this section of the support document will discuss these processes as they relate to this phenomenon of soil leaching.

B. Basic Processes Affecting Soil Leaching

The general equation (Guenzi 1974) for chemical movement through porous media under steady state soil-water flow conditions is:

$$\frac{B\delta S}{\theta\delta t} + \frac{\delta C'}{\delta t} = D' \frac{\delta^2 C'}{\delta X^2} - V \frac{\delta C'}{\delta X} \quad (1)$$

where

- B = soil bulk density (g/cm³)
- θ = volumetric water content (cm³/cm³)
- S = amount of chemical adsorbed at the soil/water interface (g/g soil).
- t = time (sec.)
- C' = solution concentration of chemical
- D' = dispersion coefficient (cm²/sec)
- V = average pore-water velocity (cm/sec)
- X = space coordinate measured normal to the section

Most mass transport equations represent simplifications of "real world" conditions. Equation 1 and similar mathematical expressions try to describe the chromatographic distribution of the chemical in the soil profile; however, they are gross simplifications of a phenomenon affected by a number of complex interacting processes including but not limited to precipitation, evaporation, evapotranspiration and hydrodynamic dispersion.

In general, chemical leaching is dependent upon three major processes: the mass transport of water (the direction and rate of water flow), diffusion, and the adsorption characteristics of the chemical in soil (Guenzi 1974). Diffusion is the transport of matter resulting from random molecular motion caused by molecular thermal energy. This random motion will lead to the uniform distribution of molecules in a closed system since there is net movement from regions of higher to lower concentrations. In this document, adsorption refers to the equilibrium distribution of a molecule between a solid phase and a solution phase. As the degree of adsorption increases, the concentration of the chemical in the soil water and the soil air decreases. This equilibrium process is governed by two opposing rate processes. The adsorption rate is the rate to which molecules from the liquid phase transfer into the adsorbed state in the solid phase. The desorption rate is the opposite process, i.e., the rate at which molecules transfer from the adsorbed state in the solid phase into the liquid phase. In general, the mass transport, diffusion, and adsorption processes produce the observed leaching pattern of a chemical in soil.

C. Chemical Properties Affecting Leaching

The main process of the three processes discussed above which determines a chemical's leaching potential (as described mathematically in equation 1) is adsorption. Adsorption is governed by the properties of both the adsorbent and the adsorbate. The important properties of the adsorbate affecting adsorption by soil colloids (Bailey and White 1970) are: (1) chemical structure and conformation (2) acidity or basicity of the molecule (pK_a or pK_b), (3) water solubility, (4) permanent charge, (5) polarity, (6) molecular size, and (7) polarizability. There are many ways in which each of these adsorbate properties interact and are manifested in the overall adsorption reaction (Bailey and White 1970).

D. Soil Properties Affecting Leaching

Soil is the unconsolidated organic and mineral material on the immediate surface of the earth which serves as a natural medium for the growth of plants. The combined actions of climate, microorganisms and macroorganisms over long periods of time on different parent geologic and biotic materials form soils that differ widely in their physical, chemical, and morphological characteristics. The wide variations in the amounts and types of clay and organic matter, soil pH, primary and secondary minerals, structure, texture, and exchange capacity create soils of

substantial heterogeneity within the United States. There are currently 10 Soil Orders, at least 43 Suborders, over 200 Great Groups and over 7,000 soil series recognized in the United States (Buckman and Brady 1969).

The soil properties affecting the adsorption and desorption of organics include organic matter content, type and amount of clay, exchange capacity, and surface acidity (Adams 1973; Bailey and White 1970; and Helling 1970). Soil organic matter is a primary soil parameter responsible for the adsorption of many pesticides. Helling (1970) lists many examples where the organic matter primarily influenced the adsorption of pesticides. Although organic matter and clay are the soil components most often implicated in pesticide adsorption, the individual effects of either organic matter or clay are not easily ascertained. Since the organic matter in most soil is intimately bound to the clay as a clay-metal-organic complex (Stevenson 1973), two major types of adsorbing surfaces are normally available to the chemical, namely, clay-organic and clay alone. Clay and organic matter function more as a unit than as separate entities and the relative contribution of organic and inorganic surfaces to adsorption will depend on the extent to which the clay is coated with organic substances. Comparative studies between known clay minerals and organic soils suggest that most, but not all, pesticides have a greater affinity for organic surfaces than for mineral surfaces (Stevenson 1973). Since typical soil studies compare soils in which both clay and organic matter increase and do not utilize multiple regression analyses to isolate the

governing parameter (Helling 1970), only generalizations concerning the relative importance of clay and organic matter in the adsorption process can be made.

The activity of protons in the bulk suspension (i.e., as measured by pH) and the activity of protons at or in close proximity to the colloidal surface (i.e., the acidity in the interfacial region) may differ significantly. The term "surface acidity" as applied to soil systems is the acidity at or in close proximity to the colloidal surface and reflects the ability of the system to act as a Lewis acid. Surface acidity is a composite term which reflects both the total number of acidic sites and their relative degree of acidity. Surface acidity is probably the most important property of the soil or colloidal system in determining the extent and nature of adsorption of basic organic chemicals as well as determining if acid-catalyzed chemical transformation occurs (Bailey and White 1970). There is overwhelming evidence, mainly from infrared studies as well as other studies, pointing to the fact that there is protonation of basic chemicals by clays having hydrogen and aluminum as the predominant exchangeable cation and by clays saturated with alkali, alkaline earth, and transition metal cations. A summary of recent investigations indicates that the protonation of chemicals in the interfacial region of clays is a function of the basicity of the molecule, the nature of the exchangeable cation on the clay, water content of the clay system, and the origin of negative charge in the aluminosilicate clay (Bailey and White 1970).

In summary, the chemical properties discussed in (C) and the soil properties discussed in (D) both govern the extent of adsorption in soils.

E. Types of Adsorptive Forces

The specific type of interaction of organic molecules with soil will depend on the specific chemical properties of the organic molecule and the type of soil. These specific interactions or adsorptive forces are usually classified as: van der Waals forces, charge transfer, ion exchange, and hydrophobic bonding (Adams 1975, Goring and Hamaker 1972).

The van der Waals forces arise from the fluctuations in a molecule's electron distribution as the electrons circulate in their orbitals. These fluctuations produce instantaneous dipoles which cause that molecule's attraction to other atoms and molecules. Charge transfer involves the formation of a donor-acceptor complex between an electron donor molecule and an electron acceptor molecule with partial overlap of their respective molecular orbitals and a partial exchange of electron density. Ion exchange refers to the exchange between counterions balancing the surface charge on the soil colloid and the ions in the soil solution. The driving force for this interaction is the requirement for electroneutrality: the surface electric charge must be balanced by an equal quantity of oppositely charged counterions. In general, ion exchange is reversible, diffusion controlled, stoichiometric and, in most cases, exhibits some

selectivity or preferential adsorption for one ion over another competing ion. Hydrophobic solvation, the process commonly referred to as hydrophobic bonding, refers to the preference of an organic molecule for a hydrocarbon solvent or hydrophobic region of a colloid over a hydrophilic solvent. This preference is due to the fact that hydrocarbon regions of a molecule have greater solubility in liquid hydrocarbons (or most organic solvents) than in water. In general, one or more of these specific interactions or adsorptive forces may occur at the same time depending on the presence and magnitude of the chemical and soil properties discussed above.

F. Surface Transformations

A special type of interaction between organic molecules and soils deals with the transformation of organic chemicals into new compounds containing different chemical structures through the catalytic activity of the soil colloid surfaces. Although several theories exist to account for the mechanism of these transformations, no scheme predicting the occurrence of such surface reactions presently exists. Therefore, it is extremely important that parent compound mass balances be performed and reported in order to ascertain the extent of such transformations during soil leaching experiments. Also, the leaching pattern (a diagram or photograph of the TLC plate showing the position of the chemical) can give a qualitative indication of the extent of such transformations and should be reported. The scientific literature shows that a number of chemicals and chemical classes

undergo colloid surface induced chemical transformations. Poly-(dimethylsiloxane) fluids in intimate contact with many soils undergo siloxane bond redistribution and hydrolysis, resulting in the formation of low molecular weight cyclic and linear oligomers (Buch and Ingebrightson 1979). S-triazines (White 1976) and organophosphorus pesticides (Yaron 1978, and Mingelgrin et al. 1977) undergo clay colloid induced hydrolysis. Benzene and phenol polymerize into high molecular weight species by adsorption and reaction at the surface of smectite saturated with transition metal cations (Mortland and Halloran 1976). Gallic acid, pyrogallol, protocatechuic acid, caffeic acid, orcinol, ferulic acid, p-coumaric acid, syringic acid, vanillic acid and p-hydroxybenzoic acid undergo oxidative polymerization in the presence of various clay minerals (Wang and Li 1977, and Wang et al. 1978). In general, testing methods that do not take into account surface transformations should not be used in determining the leaching potential of chemicals.

In summary, the interfacial region is important in determining the adsorption mechanism, the energy by which the adsorbate is held, and in determining if the adsorbed chemical is transformed. This information is important in determining the persistence and ultimate toxicity of the molecule since the transformation product(s) (1) may be more or less toxic than the original compound, (2) may be more or less tightly bound than the original compound, and (3) may have a water solubility either greater than or less than the original compound, thereby affecting its leaching and movement into the groundwater.

III. SCIENTIFIC ASPECTS OF THE TEST

A. Development of Soil Thin Layer

Chromatography (TLC)

Before 1968, methods of investigating the mobility of nonvolatile organic chemicals within soils were based on the use of field analysis, soil adsorption isotherms, and soil columns. In 1968, Helling and Turner introduced soil thin layer chromatography (soil TLC) as an alternate procedure. It is analogous to conventional TLC, with the use of soil instead of silica gels, oxides, etc. as the adsorbent phase.

In their initial report, Helling and Turner used Lakeland sandy loam, Chillum silt loam, and Hagerstown silty clay loam. Medium sand (250)µm dia.) was removed from Chillum and Hagerstown soils and coarse sand (500 µm) from Lakeland soil by dry-sieving. Aqueous slurries were prepared and 0.50 mm (silt loam, silty clay loam) or 0.75 mm (sandy loam) thick layers were spread on TLC plates using conventional TLC apparatus. After drying, six or seven radiolabelled pesticides were applied near the base of a 20 x 20 cm plate and developed ten cm with water by ascending chromatography. Pesticide movement was visualized by autoradiography. Movement was expressed by the conventional R_f designation, although this referred to the front of pesticide movement rather than its maximum concentration. The soil TLC data are most appropriately compared with other mobility data

which indicate the depth to which an organic chemical may be leached. The ranking of pesticides in order of mobility is in good agreement with general trends previously reported.

Absolute movement on soil TLC plates cannot be transposed directly to field or soil column experiments. Since soil structure in the TLC system is considerably more homogeneous than in most other systems, band spreading will be somewhat less than in field or column regimes. Flow rates are also higher than those occurring naturally. For example, infiltration into Hagerstown silty clay loam was equivalent to rainfall of about 1.2 cm/hr (Helling 1970). High flow rates are usually associated with increased mobility, as later correlations (Helling 1968) bore out. In spite of these problems, monitoring data utilizing certain reference chemicals has provided the necessary information to relate soil TLC data to column and field data. In general, Helling and Turner (1968) indicated that soil TLC offered a rapid, simple, and inexpensive procedure for establishing a general mobility classification of pesticides and organic chemicals.

Simple chromatographic theory can be used to correlate adsorption coefficients with soil TLC R_f values. If chromatographic movement through a soil column is treated according to the distillation theoretical plate theory (Block et al. 1958, Martin and Synge 1941), a formula for R_f is obtained in

terms of the relative cross-sectional areas of the liquid and solid phases and partitioning of a chemical between solid and liquid phases (Hamaker 1975):

$$R_f = A_L / (A_L + A_S) = 1 / [(1 + a (A_L + A_S))] \quad (2)$$

where A_S and A_L are cross-sectional areas of solid and liquid phases and a is the ratio of volume concentration in the solid phase to that in the liquid phase. For saturated conditions which will be assumed for a soil plate, $A_L + A_S = A$ (cross-sectional area), this can be written:

$$R_f = 1 / [(1 + a (A / [A_L - 1]))] \quad (3)$$

When reexpressed in terms of the pore fraction of the soil r , density of soil solids (d_s), and a soil adsorption coefficient K , this equation becomes:

$$R_f = (1 + K(d_s)(1/\phi^{2/3} - 1))^{-1} \quad (4)$$

This ratio, A/A_L , is set equal to $1/\phi^{2/3}$ by analogy to the treatment of soil diffusion by Millington and Quirk (1961) where it serves to correct for the tortuosity of flow through the porous medium. In this case, it serves to relate the pore volume to the cross sectional area of the liquid phase in a saturated

soil. In general, equation 4 has shown that an inverse relationship exists between the soil adsorption coefficient K and R_f (Hamaker 1975).

Riley (1976) presented a general relationship between the soil/solution distribution coefficient K and the depth of pesticide leaching. Relating the data of Riley (1976) with the R_f values of Helling (1968, 1971a, 1971b, 1971c) and the average K values of Goring and Hamaker (1972) for selected pesticides, the general relationship shown in Table 1 was developed between the soil/solution partition coefficient, R_f , and soil mobility.

B. Rationale for the Selection of Soil TLC

A number of laboratory tests - the soil thin layer chromatography, soil adsorption isotherm, and soil columns - have been developed to obtain an estimate of a chemical's leaching potential (Hamaker 1975). Soil TLC is the least expensive of the available tests which measures leaching potential, and is widely used; furthermore, it offers many desirable features. First, mobility results are reproducible. Mass transfer and diffusion components are distinguishable. The method has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It yields data that are amenable to statistical analyses. A chemical extraction-mass balance procedure to elicit information on degradation and chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease with which the R_f and mass balance are performed will depend

Table 1. The General Relationship Between the Soil/Solution
Partition Coefficient K , R_d and Soil Mobility

K	R_d	Mobility class	Distance surface applied chemical may leach
0.1.....	0.95	Very Mobile.....	
1.....	0.60		Much of chemical leaches through top 20 cm soil into subsoil.
10.....	0.25	Mobile.....	Much of chemical leached into soil but peak concentration in top 20 cm soil.
10^2	0.10	Low mobility.....	Only small amount of leaching and peak concentration normally in top 5 cm soil.
$10^{2.5}$	0.00	Immobile.....	No significant leaching.
10^3	0.00		
10^4	0.00		

upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques for measuring the chemical.

C. Rationale for Selection of Experimental
Conditions and Procedures

The papers by Helling (1968, 1971a, 1971b, 1971c) and Helling and Turner (1968) were the basis of this test guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiates the experimental conditions specified in this Test Guideline as accepted, standard procedures. A few of these conditions will be discussed in greater detail below.

Soil TLC can be used to determine the soil mobility of sparingly water soluble to infinitely soluble chemicals. In general a chemical having a water solubility of less than 0.5 ppm need not be tested since the literature indicates that these chemicals are, in general, immobile (Goring and Hamaker 1972). However, this does not preclude advanced soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

Soil TLC may be used to test the mobility of volatile chemicals by placing a clean plate over the spotted soil TLC plate and then placing both plates in a closed chromatographic chamber.

Soil TLC was originally designed for use with soils. The literature shows no published use of this method with sediments as the adsorbent phase, probably due to the fact that sediment surface properties change significantly during air drying. It is extremely important that the TLC plate with the adsorbent be air dried before leaching studies can be undertaken.

Distilled-deionized H_2O is required in order to minimize competition effects for soil exchange sites by cationic and anionic species normally present in tap and distilled H_2O .

It is extremely important that the test chemical be of the purest grade available. Impurities may produce migration patterns on the TLC plate independent of the parent chemical and may be misinterpreted as transformation products. Transformation product identification is an expensive analytical procedure that may be unnecessarily required as a result of the presence of impurities.

The sieving of soils will remove the coarse (500-2,000 μm) and medium (250-500 μm) sand fractions. Published testing results showed that removal of a portion of sand had no affect on the mobility of a test compound but aided in achieving a more cohesive uniform soil layer and more reproducible results (Helling 1971a).

Gentle crushing and grinding should be used to reduce soil aggregate size. Fine particles (silt and clay) in excess of the amount originally present may be created if excessive pressure is exerted on the aggregates.

It is important that application of the soil slurry to clean glass plates be done quickly to prevent particle size segregation. A specific method of soil slurry application was not identified since a number of methods which produce the acceptable layer thickness are in use today.

Replication of the basic experimental unit was necessary in order to estimate the standard deviation of the treatment mean. Three replicates are considered to be the minimum number of replicates for a statistically acceptable estimation. The soils literature indicates that, in general, the standard deviation should be less than $0.01 R_f$ units for soil TLC.

Since the available literature indicated that pesticide mobility on soil TLC plates did not significantly change when temperature varied from 2° to 25°C (Helling 1971a), only a room temperature range was suggested.

The Soil Order, Series, and general clay fraction mineralogy data may be found in Soil Survey Reports published after approximately 1970. Pre-1970 reports may not contain mineralogy data. Soil Survey Reports have been issued for most U.S. counties and may be obtained from County Extension Offices; the State Office of the U.S. Department of Agriculture Soil

Conservation Service; or the USDA-Soil Conservation Service,
Publications and Information Division, P.O. Box 2890, Washington,
DC 20013. If mineralogy data are not printed in a report, the
State Office of the U.S. Department of Agriculture Soil
Conservation Service may be contacted for assistance in obtaining
general clay mineral data of a particular soil.

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SEDIMENT AND SOIL ADSORPTION ISOTHERM

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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I. INTRODUCTION

A. Background and Purpose

The adsorption of chemicals to sediments and soils is an important process that affects a chemical's distribution in the environment. If a chemical is adsorbed to soil particles, it will remain on the soil surface and will not reach ground water. If a chemical is not adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Similarly, if a chemical adsorbed to sediment, it will accumulate in the bed and suspended load of aquatic systems. If a chemical is not adsorbed to sediment, it will accumulate in the water column of aquatic systems. Information on the adsorption potential is needed under certain circumstances to assess the transport of chemicals in the environment. This Test Guideline describes procedures that will enable sponsors to determine the adsorption isotherm of a chemical on sediments and soils.

B. Definitions and Units

The "cation exchange capacity" (CEC) is the sum total of exchangeable cations that a sediment or soil can adsorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meq/100g) or milliequivalents of negative charge per gram (meq/g) of soil or sediment.

"Clay mineral analysis" is the estimation or determination of the kinds of clay-size minerals and the amount present in a sediment or soil.

"Organic matter" is the organic fraction of the sediment or soil; it includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by the microbial population.

"Particle size analysis" is the determination of the various amounts of the different particle sizes in a sample (i.e., sand, silt, clay), usually by sedimentation, sieving, micrometry, or combinations of these methods. The names and diameter range commonly used in the United States are:

<u>Name</u>	<u>diameter range</u>
very coarse sand	2.0 to 1.0 mm dia.
coarse sand	1.0 to 0.5 mm
medium sand	0.5 to 0.25 mm
fine sand	0.25 to 0.125 mm
very fine sand	0.125 to 0.062 mm
silt	0.062 to 0.002 mm
clay	<0.002 mm

The "pH" of a sediment or soil is the negative logarithm to the base ten of the hydrogen ion activity of the sediment or soil suspension. It is usually measured by a suitable sensing electrode coupled with a suitable reference electrode at a 1/1 solid/solution ratio by weight.

The adsorption ratio, " K_d ," is the amount of test chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of test chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio.

"Sediment" is the unconsolidated inorganic and organic material that (a) is suspended in and being transported by surface water, or (b) has settled out and has deposited into beds.

"Soil" is the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants. Its formation and properties are determined by various factors such as parent material, climate, macro- and microorganisms, topography, and time.

"Soil aggregate" is the combination or arrangement of soil separates (sand, silt, clay) into secondary units. These units may be arranged in the soil profile in a distinctive characteristic pattern that can be classified according to size, shape, and degree of distinctness into classes, types, and grades.

"Soil classification" is the systematic arrangement of soils into groups or categories. Broad groupings are based on general soil characteristics while subdivisions are based on more detailed differences in specific properties. The soil classification system used in this standard and the one used today in the United States is the 7th Approximation-Comprehensive System. The ranking of subdivisions under this system is: Order, Suborder, Great group, family, and series.

A "soil horizon" is a layer of soil approximately parallel to the land surface. Adjacent layers differ in physical, chemical, and biological properties such as color, structure, texture, consistency, kinds and numbers of organisms present, and degree of acidity or alkalinity.

"Soil Order" is the broadest category of soil classification and is based on the general similarities of soil physical/chemical properties. The formation of soil by similar general genetic processes causes these similarities. The Soil Orders found in the United States are: Alfisol, Aridisol, Entisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol, and Vertisol.

"Soil series" is the basic unit of soil classification and is a subdivision of a family. A series consists of soils that were developed under comparable climatic and vegetational conditions. The soils comprising a series are essentially alike in all major profile characteristics except for the texture of the "A" horizon (i.e., the surface layer of soil).

"Soil texture" is a classification of soils that is based on the relative proportions of the various soil separates present. The soil textural classes are: clay, sandy clay, silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand.

C. Principle of the Test Method

The extent of adsorption of a chemical onto sediment or soil is measured, using this test guideline, by equilibrating aqueous solutions containing different, but environmentally realistic, concentrations of the test chemical with a known quantity of sediment or soil. After equilibrium is reached, the distribution of the chemical between the water phase and the solid phase is quantitatively measured by a suitable analytical method. Then, sorption constants are calculated by using the Freundlich equation:

$$x/m = C_s = KC_e^{1/n} \quad (1)$$

where

- C_e = Equilibrium concentration of the chemical in the solution phase
- C_s = Equilibrium concentration of the chemical in the solid phase
- K = Freundlich adsorption coefficient
- m = The mass of the solid in grams
- $1/n$ = Exponent where n is a constant
- x = The mass in micrograms of the chemical adsorbed by m grams of solid.

Logarithmic transformation of the Freundlich equation yields the following linear relationship:

$$\log C_s = \log K + (1/n) \log C_e \quad (2)$$

In order to estimate the environmental movement of the test chemical, the values K and $1/n$ are compared with the values of other chemicals whose behavior in soil and sediment systems is well-documented in scientific literature.

The adsorption isotherm (AI) test has many desirable features. First, adsorption results are highly reproducible. The test provides excellent quantitative data readily amenable to statistical analyses. Also, it has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It allows solution phase organic chemical determinations that are relatively uncomplicated. A chemical extraction-mass balance procedure to elicit information on chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease of performing the isotherm test and mass balance will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques to measure the chemical.

The papers by Aharonson and Kafkafi (1975), Harvey (1974), Murray (1975), Saltzman (1972), Weber (1971), and Wu (1975) served as the basis for this Test Guideline. The soil and colloid

chemistry literature and the analytical chemistry literature substantiate the experimental conditions and procedures specified in this guideline as accepted, standard procedures.

D. Applicability and Specificity

The AI Test Guideline can be used to determine the soil and sediment adsorption potential of sparingly water soluble to infinitely soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested with soil as the solid phase, since the literature indicates that these chemicals are, in general, immobile in soils. (Goring and Hamaker, 1972). However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

II. TEST PROCEDURES

A. Test Conditions

1. Special laboratory equipment

- a. Equilibrating solutions that contain, besides the test chemical, 0.01M calcium nitrate dissolved in sterilized, distilled-deionized H₂O adjusted to neutral pH 7 by boiling to remove CO₂.
- b. Containers that are composed of material that (1) adsorb negligible amounts of test chemical, and (2) withstand high speed centrifugation. The volume of the container is not a major consideration; however, it is extremely important that the amount of soil or sediment and the solid/solution ratio used in the study result in minimal container headspace. It is also extremely important that the containers be sterilized before use.
- c. A 150 m (100 mesh) stainless steel or brass sieve.

- d. Drying oven, with circulating air, that can attain 100°C.
- e. Vortex mixer or a comparable device.
- f. Rotary shaker or a comparable device.
- g. High speed temperature-controlled centrifuge capable of sedimenting particles greater than 0.5 μ m from aqueous solution.

2. Temperature

It is recommended that the test procedure be performed at 23 $\pm$ 5°C.

3. Replications

It is recommended that three replications of the experimental treatments be used.

4. Soil Pretreatment

It is extremely important that these soil pretreatment steps be performed under the following conditions:

- a. Decrease the water content, air or oven dry soils at or below 50°C.

- b. Reduce aggregate size before and during sieving, crush and grind dried soil very gently.
- c. Eliminate microbial growth during the test period using a chemical or physical treatment that does not alter or minimally alters the soil surface properties.
- d. Sieve soils with a 100 mesh stainless steel or brass sieve.
- e. Store all solutions and soils at temperatures between 0 and 5°C.

5. Sediment Pretreatment

It is extremely important that these sediment pretreatment steps be performed under the following conditions:

- a. Decrease the H₂O content by air or oven drying sediments at or below 50°C. Sediments should not be dried completely and should remain moist at all times prior to testing and analysis.
- b. Eliminate microbial growth during the test period by using a chemical and/or physical treatment that does not alter or minimally alters the colloid surface's properties.

- c. Store at temperatures between 0 and 5°C.

6. Solid/Solution Ratio

It is recommended that the solid/solution ratio be equal to or greater than 1/10. If possible, the ratios should be equal to or greater than 1/5. The sediment or soil dry weight after drying for a 24 hour minimum at 90°C is recommended for use as the weight of the solid for ratio and data calculations.

7. Equilibration Time

The equilibration time will depend upon the length of time needed for the parent chemical to attain an equilibrium distribution between the solid phase and the aqueous solution phase. It is recommended that the equilibration time be determined by the following procedure:

- a. Equilibrate one solution containing a known concentration of the test chemical with the sediment or soil in a solid/solution ratio not exceeding 1/10 and preferably equal to or greater than 1/5. It is important that the concentration of the test chemical in the equilibrating solution (1) does not exceed one half of its solubility and (2) should be 10 ppm or less at the end of the equilibration period.

- b. Measure the concentration of the chemical in the solution phase at frequent intervals during the equilibration period.
- c. Determine the equilibration time by plotting the measured concentration versus time of sampling; the equilibration time is the minimum period of time needed to establish a rate of change of solution concentration of 5 percent or less per 24 hours.

8. Centrifugation Time

Calculate the centrifugation time, t_c , necessary to remove particles from solution greater than approximately 0.5 μ m (5×10^{-5} m) equivalent diameter (which represents all particles except the fine clay fraction) using the following equation:

$$t_c(\text{min}) = 1.41 \times 10^9 [\log(R_2/R_1)]/N^2 \quad (3)$$

where

t_c = centrifuge time in minutes

R_2 = distance from centrifuge spindle to deposition surface of centrifuge

R_1 = distance from spindle to surface of the sample

N = number of revolutions of the centrifuge per minute.

9. Storage of Solutions

If the chemical analysis is delayed during the course of the experiment, store all solutions between 0 and 5°C.

10. Solvents for Extraction

It is extremely important that (1) the purity of the solvent used to extract the chemical that is adsorbed on the sediment or soil is analytical grade or better and (2) the minimum solubility of the test chemical in the solvent is 10 g/l.

B. Test Procedure

1. Equilibration

Add six solutions containing different concentrations of the test chemical to at least one gram of each solid. The initial concentration of the test chemical in these solutions will depend on the affinity the chemical has for the sediment or soil. Therefore, after equilibrium is attained, it is extremely important that the highest concentration of the test chemical in the equilibrating solution (a) does not exceed 10 ppm, (b) is at least one order of magnitude greater than the lowest concentration reported, and (c) does not exceed one half of its solubility.

- a. Immediately after the solutions are added to the solids, tightly cap the containers and vigorously agitate them for several minutes with a vortex mixture or similar device.
- b. Shake the containers throughout the equilibration period at a rate that suspends all solids in the solution phase.

2. Centrifugation

When the equilibration time has expired, centrifuge the containers for t_c minutes.

3. Chemical Extraction

- a. After centrifugation, remove the supernatant aqueous phase from the solid-solution mixture.
- b. Extract the chemical adsorbed on the sediment or soil colloid surfaces with solvent.

4. Chemical Analysis

Determine the amount of parent test chemical in the aqueous equilibrating solution and organic solvent extractions. Use any method or combination of methods suitable for the identification and quantitative detection of the parent test chemical.

III. REPORTING

Report the following information.

- (1) Temperature at which the test was conducted.
- (2) Detailed description of the analytical technique(s) used in the chemical extraction, recovery, and quantitative analysis of the parent chemical.
- (3) Amount of parent test chemical applied, the amount recovered, and the percent recovered.
- (4) Extent of adsorption by containers and the approach used to correct the data for adsorption by containers.
- (5) The individual observations, the mean values, and graphical plots of x/m as a function of C_e for each sediment or soil for (a) the equilibration time determination, and (b) the isotherm determination.
- (6) The quantities K , n , and $1/n$.
- (7) Soil information: Soil Order, series, texture, sampling location, horizon, general clay fraction mineralogy.

- (8) Sediment information: sampling location, general clay fraction mineralogy.
- (9) Sediment and soil physical-chemical properties: percent sand, silt, and clay (particle size analysis); percent organic matter; pH (1/1 solids/H₂O); and cation exchange capacity.
- (10) The procedures used to determine the physical/chemical properties listed above.

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SEDIMENT AND SOIL ADSORPTION ISOTHERM

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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SEDIMENT AND SOIL ADSORPTION ISOTHERMI. NEED FOR THE TEST

The Sediment and Soil Adsorption Isotherm (AI) is a screening test suitable for obtaining an estimate of the sediment adsorption potential of a chemical and its soil leaching potential. The adsorption affects the distribution of a chemical in the environment. Knowledge of the adsorption potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it may leach through the soil profile and may reach ground waters and then surface waters. Similarly, if a chemical is tightly adsorbed to sediment, it will accumulate in the bed and suspended load of aquatic systems. If a chemical is weakly adsorbed to sediment, it may be found predominately in the water column of aquatic systems.

Since adsorption can affect the distribution of a chemical in the environment, it may have a profound effect on a chemical's effect on man, the ecosystem in question, and on species within the ecosystem. If a chemical reaches ground and/or surface waters, it may cause deleterious human health effects by contaminating the drinking water. If a chemical remains at the soil surface, it may cause deleterious environmental and human health effects by contaminating the drinking water. If a chemical remains at the soil surface, it may cause deleterious environmental and human health effects due to its presence in the zone of plant growth that may result in contaminated feed and food.

II. SCIENTIFIC ASPECTS OF SOIL LEACHING

A. Introduction

The leaching of chemicals in soils is affected by several interacting processes, including adsorption, that occur at the soil-water interface. The interfacial region is important for two reasons. First, it determines the adsorption mechanism and the energy by which the chemical is held. Second, it may catalyze the transformation of the original compound. Transformation product(s) are of particular concern, since they (1) may be toxic to a greater or lesser degree than the original compound, (2) may be absorbed either greater than or less than the original compound, and (3) may have a water solubility either greater than or less than the original compound, thereby affecting its leaching and movement into the ground water. This section of the support document will discuss these processes as they relate to leaching and the AI Test Guideline.

B. Basic Processes Affecting Soil Leaching

The leaching of chemicals through soil is a complex phenomenon consisting of several major processes (Hamaker 1975). One general equation (Guenzi 1974) for chemical movement through porous media under steady state soil-water flow conditions for water in soil is:

$$\partial S / \partial t + \partial c' / \partial t = D' \partial^2 c' / \partial x^2 - v \partial c' / \partial x \quad (1)$$

where B = soil bulk density (g/cm^3)
 θ = volumetric water content (cm^3/cm^3)
 S = mass fraction of test chemical adsorbed at the
 soil/water interface ($\text{g test chemical}/\text{g soil}$)
 t = time (s)
 c' = concentration of test chemical in solution (g/cm^3)
 D' = dispersion coefficient (cm^2/s)
 V = average pore-water velocity (cm/s)
 X = space coordinate measured normal to the section

Most mass transport equations represent simplifications of "real world" conditions that attempt to describe the chromatographic distribution of the chemical in the soil profile. They are gross simplifications of a phenomenon that is affected by complex, interacting processes. In general, chemical leaching is dependent upon three major processes: the mass transport of water (the direction and rate of water flow), the rate of diffusion, and the adsorption characteristics of the chemical in soil (Guenzi 1974).

Diffusion is the transport of matter resulting from random molecular motion, which is caused by molecular thermal energy. This random motion leads to the uniform distribution of molecules in a closed fluid system, since a net movement of molecules from regions of higher to lower concentrations occurs.

Adsorption is the accumulation of molecules by the attractive forces of the surface of a solid phase. When adsorption is a

significant factor, there is a higher concentration of a chemical in an extremely thin layer at the surface of a sediment or soil than is present in the bulk aqueous solution associated with the sediment and soil. The equilibrium adsorption-desorption process is governed by two opposing rate processes. The adsorption rate is the rate at which molecules from the liquid phase transfer into the adsorbed state in the solid phase and the rate increases as the concentration of dissolved species increases. The desorption rate is the rate of the opposite process, i.e., the rate at which molecules transfer from the adsorbed state in the solid phase into the liquid phase. Equilibrium is established when the rates of these two processes are equal.

C. Chemical Properties Affecting Adsorption

Adsorption is the main process that determines a chemical's leaching potential as described mathematically in equation 1. It is governed by the properties of both the solid phase and the adsorbate. The important properties of the adsorbate that affect adsorption by soil (Bailey and White 1970) are: (1) chemical structure and conformation (2) molecular size, (3) acidity or basicity of the molecule (pK_a or pK_b), (4) water solubility, (5) permanent charge, (6) polarity, and (7) polarizability.

D. Soil Properties Affecting Adsorption

The soil properties affecting the adsorption and desorption of organics include the organic matter content, type and amount of

clay, exchange capacity, and surface acidity (Adams 1973, Bailey and White 1970, and Helling 1970). The combined actions of climate, micro-, and microorganisms over long periods of time on different parent geologic and biotic materials form soils that differ widely in their physical, chemical, morphological, and adsorption characteristics. The amounts and types of clay and organic matter, soil pH, primary and secondary minerals, structure, texture, and exchange capacity vary considerably for U.S. soils. There are currently 10 Soil Orders, at least 43 Suborders, over 200 Great groups and over 7,000 soil series recognized in the United States (Buckman and Brady 1969).

Soil organic matter is a primary soil property responsible for the adsorption of many chemicals. Helling (1970) lists many examples where the organic matter primarily influenced the adsorption of pesticides. Organic matter and clay are the soil components most often implicated in pesticide adsorption. However, the individual effects of either organic matter or clay are not easily ascertained. Since the organic matter in most soil is intimately bound to the clay as a clay-metal-organic complex (Stevenson 1976), two major types of adsorbing surfaces are normally available to the chemical, namely, clay-organic and clay alone. Clay and organic matter function more as a unit than as separate entities and the relative contribution of organic and inorganic surfaces to adsorption will depend on the extent to which the clay is coated with organic substances. Comparative studies between known clay minerals and organic soils suggest that most, but not all, pesticides have a greater affinity for organic

surfaces than for mineral surfaces (Stevenson 1973). Since typical studies compare soils in which both clay and organic matter increase and do not use multiple regression analyses to isolate the governing parameter (Helling 1970), only generalizations concerning the relative importance of clay and organic matter can be made.

Surface acidity is another soil property affecting the adsorption of many organic chemicals. Surface acidity is probably the most important property of the soil or colloidal system in determining the extent and nature of adsorption of basic organic chemicals, as well as determining if acid-catalyzed chemical transformation occurs (Bailey and White 1970). The activity of protons in the bulk suspension, which is expressed by pH, and the activity of protons at or in close proximity to the colloidal surface (i.e., the acidity in the interfacial region) may differ significantly. The term "surface acidity," when applied to soil systems, is the acidity at or in close proximity to the colloidal surface that reflects the ability of the system to act as a Lewis acid. Surface acidity is a composite term that reflects both the total number of acidic sites and their relative degree of acidity.

Overwhelming evidence, mainly from infrared and other studies, indicates that the clays that protonate basic chemicals either have hydrogen and aluminum as the predominant exchangeable cations, or are saturated with alkali metal, alkaline earth metal, and transition metal cations. A summary of recent investigations indicates that the protonation of chemicals in the interfacial

region of clays is a function of the basicity of the molecule, the nature of the exchangeable cation on the clay, water content of the clay system, and the origin of negative charge in the aluminosilicate clay (Bailey and White 1970).

E. Types of Adsorptive Forces

The specific type of interaction that organic molecules have with soil depends on the chemical properties of the organic molecules and the type of soil. These interactions or adsorptive forces are classified as: van der Waals forces, charge transfer, ion exchange, and hydrophobic bonding (Adams 1975, Goring and Hamaker 1972). In general, one or more of these specific interactions or adsorptive forces may occur simultaneously.

The attractive van der Waals forces or polarizability forces arise from the random fluctuations in a molecule's electron distribution. These fluctuations theoretically produce instantaneous dipoles due to the concentration of charges in one region of the molecule and cause that molecule's attraction to other atoms and molecules.

Charge transfer involves the formation of a donor-acceptor complex between an electron donor molecule and an electron acceptor molecule with partial overlap of their respective molecular orbitals and a partial exchange of electron density.

Ion exchange refers to the exchange between counterions balancing the surface charge of the soil colloid and the ions in

the soil solution. The driving force for this interaction is the requirement for electroneutrality: the surface charge must be balanced by an equal quantity of oppositely charged counterions. In general, ion exchange is reversible, diffusion controlled, and stoichiometric in most cases. It exhibits some selectivity or preferential adsorption for one ion over another competing ion.

Hydrophobic bonding refers to the greater affinity of an organic molecule for a hydrocarbon solvent or hydrophobic region of a colloid than for a hydrophilic solvent. Hydrocarbon regions of a molecule have greater solubility in liquid hydrocarbons (or most organic solvents) than in water.

F. Surface Transformations

A special type of interaction between organic molecules and soils deals with the transformation of organic chemicals into new compounds containing different chemical structures through the catalytic activity of the soil colloid surfaces. Although several theories exist to account for the mechanism of these transformations, no scheme that predicts the occurrence of these surface reactions presently exists. Therefore, it is extremely important that mass balance calculations for the test chemical are performed to ascertain the extent of these transformations during soil leaching experiments.

The scientific literature shows that a number of chemicals and chemical classes undergo colloid surface induced chemical transformations. Poly-(dimethylsiloxane) fluids in intimate

contact with many soils undergo siloxane bond redistribution and hydrolysis that result in the formation of low molecular weight cyclic and linear oligomers (Buch and Ingebrightson 1979). Substituted (White 1976) and organophosphorus pesticides (Yaron 1978, Mingelrin et al. 1977) undergo clay colloid induced hydrolysis. Benzene and phenol polymerize into high molecular weight chemicals by adsorption and reaction at the surface of smectite saturated with transition metal cations (Mortland and Halloran 1976). Gallic acid, pyrogallol, protocatechuic acid, caffeic acid, orcinol, ferulic acid, p-coumaric acid, syringic acid, vanillic acid, and p-hydroxybenzoic acid undergo oxidative polymerization in the presence of various clay minerals (Wang and Li 1977, Wang et al. 1978).

III. SCIENTIFIC ASPECTS OF SEDIMENT-CHEMICAL TRANSPORT AND ADSORPTION

A. Introduction

The transport and adsorption of chemicals by sediment are affected by a large number of interacting processes. Furthermore, chemical movement in aquatic systems, unlike soil systems, is dependent not only on the extent of adsorption but on the movement of sediment. This Test Guideline will develop data on the extent of a chemical's adsorption onto sediments. Sediment movement can be mathematically estimated in several ways for a specific situation provided hydrologic and meteorologic information is available. This section of the support document will discuss the transport and adsorption of chemicals by sediment as they relate to the Test Guideline.

B. Basic Sedimentation Processes Affecting Chemical Movement

Sediment is the unconsolidated inorganic and organic material that is being transported or has been transported by and deposited in beds from water. Synthesis, erosion, transportation, and deposition of sediment are natural processes that have occurred throughout geologic time. The extent of biologic activity and the extent of erosion will govern the amount of sediment that enters a watershed (Chow 1964). In general, every sediment particle that passes a particular cross section of a water body must satisfy two

conditions: (a) it must have been eroded or synthesized somewhere in the watershed above the cross section and (b) it must have been transported by the flow of water from the place of erosion or synthesis to the cross section. (Chow 1964). The wash load is the finer sediment fraction that the flow can easily carry in large quantities. The bed-material load is the coarser sediment fraction that is difficult to move by the flow and is limited in its movement by the transporting ability of the flow between source and section. The bed load is the sediment in the bed layer that cannot be suspended in the water column for fluid-dynamic reasons. The basic difference between wash load and bed-material load can best be visualized in a concrete-lined channel. If the flow is large and fast, the flow condition is not in any way affected by adding small amounts of a fine and easily transported material. This added material, the wash load, moves in suspension with the flow at the same average velocity and does not settle. If the flow velocity and discharge are now reduced and/or if the material is increased in size and rate, sediment will begin to deposit on the channel bottom and a granular sediment bed will develop. These sedimenting particles are designated as the bed-material load.

Sediment particle size is the single most important physical parameter affecting sediment transport and deposition. The exemplified differences between wash load, bed-material load, and bed load show that different sediment sizes behave differently in the same hydrologic system. Similar quantitative differences in behavior exist between different sizes of the bed-material load.

The grain size distributions in the bed and in transport are quite often different, even within the size range of the bed-material load. Also, many streams have heterogeneous beds with individual bars having very different composition and appearance. Finally, many stream and river beds exhibit another type of segregation in which all coarse particles are concentrated in lenses, or layers, at a greater or lesser depth below the bed surface.

C. Chemical Properties Affecting Adsorption

A chemical's adsorption potential is governed by the properties of both the sediment phase and the adsorbate. The important properties of the adsorbate affecting adsorption onto sediments are basically the same properties affecting adsorption onto soil colloids as discussed in Section II.C. They are: (1) chemical structure and conformation, (2) acidity or basicity of the molecule (pK_a or pK_b), (3) water solubility, (4) permanent charge, (5) polarity, (6) molecule size, and (7) polarizability.

D. Sediment Properties Affecting Adsorption

The inorganic chemical composition of sediments includes most primary and secondary minerals. In general, it is composed of mineral fragments having particle sizes ranging from clay and silt to sand, gravel, and boulders. The mineralogy of original source rocks, together with chemical weathering processes, mechanical weathering processes, and precipitation processes, determine the ultimate size, weight, shape, and, therefore, the adsorptive

capacity of inorganic sediment particles. The mineral content contributes to the sorting phenomena. Heavy minerals will deposit at higher flow velocities compared to lighter materials of equal size. Hard minerals, such as quartz, will resist abrasion to a greater degree than soft, chemically unstable minerals, such as gypsum or limestone. Some minerals and rocks disintegrate along crystal faces or cleavage planes to form platelike particles while others form equidimensional particles.

The organic sediment particles are composed of plant and animal tissue in various stages of chemical and microbial decomposition. The highly varied composition and the continuous fluctuation of the hydrologic conditions found in many aquatic systems create heterogeneous sediments with a widely range of characteristics.

A review of the literature on the adsorption of chemicals reveals that pesticide-soil studies dominate the literature. Most of the principles applicable to the adsorption and transformation of chemicals in soils (Section II, Parts C, D, E, and F) are applicable to sediments. In general, sediments have a finer texture than soils. They contain more amorphous organic matter due to the biotic activity in water and contain more clay due to the erodability of the finer soil components. Therefore, sediments generally show higher sorption tendencies than soils (Pionke and Chesters 1973, Lotse et al. 1968). However, Pionke and Chesters (1973) state that little information exists on sediment-chemical interactions to indicate differences from typical soil-chemical interactions.

IV. SCIENTIFIC ASPECTS OF THE TESTA. Development of the Adsorption Isotherm

The fact that solids can remove salts and color from solution by adsorption has been known from the earliest times. For example, Aristotle knew that seawater lost some of its taste by filtration through sand (Forrester and Giles 1971b). Since then, the phenomenon of adsorption has been used to solve various water purification problems. In the 17th and 18th centuries, seawater purification by soil was used on the Barbary Coast to produce fresh water (Forrester and Giles 1971b). During the 19th century, a number of soil chemists published studies on the adsorption of both basic and acidic compounds onto soils. In 1881, van Bemmelen published the first solute-solid adsorption diagram based on his studies of sulphuric acid adsorption by metastannic acid (Forrester and Giles 1972). The only previously published adsorption diagrams were those for gas-solid adsorption plotted by Chappius and by Kayser in 1880. The term "adsorption" appeared for the first time in the English language in an 1882 abstract of Kayser's work in Nature (Forrester and Giles 1971a). The term "isotherm" was first used by Ostwald in 1885 to describe the plot of pressure (abscissa) versus the amount of gas adsorbed (ordinate) (Forrester and Giles 1971a). Soon afterwards, the term "adsorption isotherm" was adopted to describe solute-solid adsorption diagrams. In the first decade of this century, adsorption isotherms began appearing frequently in the published literature, often as adjuncts to other investigations. Since then, the

isotherm has been used for innumerable adsorption studies using an extremely wide variety of solutes, solvents, and solids over the entire range of experimentally obtainable pressures and temperatures.

Since adsorption is the major retention mechanism for most organic and inorganic compounds in soils (Section II), the magnitude of any mathematical or empirical estimation of a chemical's soil leaching potential (e.g., equation (1)) is, in general, proportional to the magnitude of the adsorption coefficient. Therefore, one need only estimate the adsorption coefficient to obtain an estimate of a chemical's leaching potential in soil. Similarly, a chemical's distribution in aquatic system will, in general, depend upon the magnitude of its adsorption to sediment. Therefore, an estimate of the adsorption potential is needed to estimate a chemical's distribution in the water column.

B. Rationale for the Selection of the Adsorption Isotherm Test

Soil thin layer chromatography, soil AI, and soil columns have been developed to obtain an estimate of a chemical's leaching potential (Hamaker 1975). The AI test is applicable to obtaining both sediment adsorption potential data and soil adsorption and mobility data.

This Test Guideline is developed upon the basic principles comprising most adsorption isotherm tests found in scientific literature. The extent of adsorption of a chemical onto sediments

or soils is measured by equilibrating aqueous solutions containing different but environmentally realistic concentrations of the test chemical with a known quantity of sediment or soil. After equilibrium is reached, the distribution of the chemical between the water phase and the solid phase is quantitatively measured by a suitable analytical method. Then, sorption constants are calculated by using the Freundlich equation:

$$x/m = C_s = KC_e^{1/n} \quad (1)$$

where

C_e =equilibrium concentration of the chemical in the solution phase

C_s =equilibrium concentration of the chemical in the solid phase

K =Freundlich adsorption coefficient

m =the mass of the solid in grams

$1/n$ =exponent where n is a constant

x =the mass in micrograms of the chemical adsorbed by m grams of solid.

A logarithmic transformation of the Freundlich equation yields the following linear relationship:

$$\log C_s = \log K + (1/n) \log C_e \quad (2)$$

In order to estimate the environmental movement of the chemical in surface water systems and soils, the values K and $1/n$ are compared with the values of other chemicals whose behavior in soil and sediment systems is well-documented in scientific literature.

The adsorption isotherm (AI) test was selected because it contains many desirable features. First, adsorption results are highly reproducible. The test provides excellent quantitative data readily amenable to statistical analyses. Also, it has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It allows solution phase organic chemical determinations that are relatively uncomplicated. A chemical extraction-mass balance procedure to elicit information on chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease of performing the isotherm test and mass balance will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques to measure the chemical.

C. Rationale for the Selection of Experimental Conditions and Procedures

The papers by Aharonson and Kafkafi (1975), Harvey (1974), Murray (1975), Saltzman (1972), Weber (1971), and Wu (1975) served as the basis for this Test Guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiate the experimental conditions and procedures specified in the suggested Test Guideline as accepted, standard procedures. The rationale for the selection of these conditions and procedures will be discussed in greater detail below.

The AI Test Guideline can be used to determine the soil adsorption potential of sparingly water soluble to infinitely water soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested, since the literature indicates that these chemicals are, in general, immobile (Goring and Hamaker 1972). However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

The 0.01M calcium nitrate is required to insure colloid flocculation during the experiment.

Distilled-deionized H_2O and glassware are required to minimize competition effects for exchange sites by unidentified cationic and anionic species normally present in tap and distilled H_2O .

Sterile water and glassware are required to minimize the potential for microbial growth in the test containers.

The absorption isotherm standard can be used to determine the adsorption potential of volatile chemicals, since the standard requires the use of containers that are capped and the use of a solid/solution ratio that minimizes container headspace.

A room temperature range of $23 \pm 5^\circ C$ was adopted since the available literature indicated that pesticide adsorption in general does not significantly change in the temperature range of 1° to $30^\circ C$.

Replication of the basic experimental unit was necessary in order to estimate the standard deviation of the treatment mean. Three replicates are considered to be the minimum number of replicates for a statistically acceptable estimation.

It is important that gentle crushing and grinding be used to reduce soil aggregate size. Fine particles (silt and clay) in excess of the amount originally present may be created if excessive pressure is exerted on the aggregates.

Soil sieving with a 100 mesh stainless steel or brass sieve is required to remove debris and coarse fragments. Removal of these components should improve the reproducibility of the adsorption test and will aid in obtaining a more uniform sample.

In order to obtain reproducible results, it is necessary to: (1) sterilize the soil or sediment to prevent microbial growth and degradation of the test chemical; (2) choose the appropriate sterilization technique to make sure that the soil or sediment surface properties are not altered; and (3) store the pretreated soil or sediment at 0 to 5°C to minimize microbial effects.

It is extremely important that the solvent chosen for extraction be: (1) analytically pure or better; and (2) the minimum solubility of the test chemical in the solvent be at least 10 g/l. The first condition is necessary to minimize the effects of solvent impurities on the analytical determination of the concentration of chemical on the soil or sediment and in aqueous solution. The second condition is necessary to make sure that the

test chemical is essentially completely extracted so that an accurate value of the concentration of chemical adsorbed on the soil or sediment and in aqueous solution is obtained.

In carrying out the adsorption experiments, it is recommended that the solid/solution ratio be approximately equal to 1/10 or greater. This ratio will give the best reproducible results. However, for certain chemicals which do not adsorb readily, it is preferable to use the ratio of approximately 1/5 or slightly greater to give the best reproducible results. However, it is not practical to use ratios much greater than 1/5, since the mixture would be too viscous and true equilibrium would not be achieved. Hence, under these conditions, the experimental results would be erroneous.

In general, a majority of chemicals should attain equilibrium between the solid and solution phases within 24 to 48 hours. For unknown reasons, however, many chemicals require longer equilibration periods. Since it is not presently possible to predict the proper equilibration period for a chemical, the Test Guideline recommends that the equilibration period be determined as a part of the test procedure for all chemicals.

In general, the test chemical should be of the purest grade readily available. Impurities may produce mass balance data indicating the presence of transformation products. Transformation product identification is an expensive analytical procedure that may be unnecessarily required due to the presence of impurities.

The Soil Order, series, and general clay fraction mineralogy data may be found in Soil Survey Reports published after approximately 1970. Pre-1970 reports may not contain mineralogy data. Soil survey reports have been issued for most U.S. counties and may be obtained from County Extension Offices; the State Office of the U.S. Department of Agriculture Soil Conservation Service; or the USDA-Soil Conservation Service, Publications and Information Division, P.O. Box 2890, Washington, DC 20013. If mineralogy data are not printed in a report, the State Office of the U.S. Department of Agriculture Soil Conservation Service may be contacted for assistance in obtaining general clay mineral data of a particular soil. The Test Guideline does not require soil mineral analysis since general clay mineralogy data may already exist for the test soil.

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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHTI. INTRODUCTIONA. Background and Purpose

Numerous chemicals enter natural aquatic systems from a variety of sources. For example, chemical wastes are discharged directly into natural water bodies, and chemicals leach into natural water bodies from landfills. Pesticides are applied directly into water bodies, and are applied to soils and vegetation and subsequently leach into water bodies. Pollutants present in aqueous media can undergo photochemical transformation in the environment (i.e., in sunlight by direct photolysis or by sensitized photolysis). As a result, there is considerable interest in photolysis in solution, especially the photolysis of pesticides. However, most of these studies have been qualitative in nature and involved the identification of photolysis products. Quantitative data in the form of rate constants and half-lives are needed to determine the importance of photochemical transformation of pollutants in aqueous media. This Test Guideline describes a two-tiered screening level approach for determining direct photolysis rate constants and half-lives of chemicals in water in sunlight.

B. Definitions and Units

- (1) "Radiant energy", or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.
- (2) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_0) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ . Thus, $A_\lambda = \log_{10} (I_0/I)$.
- (3) The "Beer-Lambert law" states that the absorbance of a solution of a given chemical species, at a fixed wavelength, is proportional to the thickness of the solution (ℓ), or the light pathlength, and the concentration of the absorbing species (C).
- (4) "Molar absorptivity (ϵ_λ)" is defined as the proportionality constant in the Beer-Lambert law when the concentration is given in terms of moles per liter (i.e., molar concentration). Thus,

$$A_\lambda = \epsilon_\lambda C \ell$$
 where A_λ and ϵ_λ represent the absorbance and molar absorptivity at wavelength λ and ℓ and C are defined in (3). The units of ϵ_λ are molar⁻¹ cm⁻¹. Numerical values of molar absorptivity depend upon the nature of the absorbing species.

- (5) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.
- (6) The "half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical being tested to be reduced to one-half its initial value.
- (7) The "sunlight direct aqueous photolysis rate constant (k_{pE})" is the first-order rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical dissolved in a water body in sunlight.
- (8) The "solar irradiance in water (L_λ)" is related to the sunlight intensity in water and is proportional to the average light flux (in the units of 2.303×10^{-3} einsteins $\text{cm}^{-2} \text{day}^{-1}$) that is available to cause photoreaction in the wavelength interval λ over a 24-hour day at a specific latitude and season date.
- (9) "The Grotthus-Draper law", the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.
- (10) The "Stark-Einstein law", the second law of photochemistry, states that only one molecule is activated to an excited state per photon or quantum of light absorbed.

- (11) The "reaction quantum yield (ϕ_λ)" for an excited state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ . It is the ratio of the number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ .
- (12) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

As a convenient reference, a glossary of the important symbols used in this Test Guideline is given in Section IV of the Support Document [CS-6000].

C. Principle of the Test Method

This Test Guideline is based on the principles developed by Zepp and Cline (1977), Zepp (1978), the U.S. Environmental Protection Agency, Office of Toxic Substances [USEPA (1979)], Mill et al. (1981, 1982a, 1982b), and Dulin and Mill (1982).

Zepp and Cline (1977) published a paper on the rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water

body. It has been shown that for dilute solution and shallow depths, the kinetics of direct photolysis can be described by the following equations:

$$\ln (C_0/C) = k_{pE} t \quad (1)$$

$$t_{1/2E} = 0.693/k_{pE} \quad (2)$$

$$k_{pE} = \phi_E k_a \quad (3)$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, $k_a = \sum k_{a\lambda}$, the sum of $k_{a\lambda}$ values of all wavelengths of sunlight that are absorbed by the chemical, t is the time, C_0 and C are the concentrations of chemical at $t = 0$ and t , and $t_{1/2E}$ represents the half-life. The term k_{pE} represents the first-order photolysis rate constant for a water body in sunlight in the units of reciprocal time.

Furthermore, under the same conditions cited above, the first-order direct photolysis rate constant, k_{pE} , is given by the equation

$$k_{pE} = \phi_E \sum \epsilon_{\lambda} L_{\lambda} \quad (4)$$

where ϕ_E is the quantum yield, ϵ_{λ} is the molar absorptivity in the units $\text{molar}^{-1} \text{cm}^{-1}$, L_{λ} is the solar irradiance in water in the units of $2.303 \times 10^{-3} \text{ einsteins cm}^{-2} \text{ day}^{-1}$ [Mill et al. (1982a)], and the summation is taken over the range

$\lambda = 290$ to 800nm . L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this guideline measures rate constants in tubes (e.g., $13 \times 100\text{mm}$) and the rate is faster in tubes. This is discussed in more detail in Section II.B.j. Thus, equations 1 and 2 have to be modified to take this into account. For simplicity, the following nomenclature is used. For water bodies, the rate constant is designated as k_{pE} with the subscript E designating rates in the environment in water bodies. For tubes, the rate constant is designated as k_p . The corresponding half-lives for water bodies and tubes are $t_{1/2E}$ and $t_{1/2}$ respectively. Thus, for tubes, equations 1 and 2 can be written as

$$\ln(C_0/C) = k_p t \quad (5)$$

$$t_{1/2} = \frac{0.693}{k_p} \quad (6)$$

A simple first-tier screening test has been developed using equation 4. As an approximation, it is assumed that the quantum yield ϕ_E is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 4 becomes

$$(k_{pE})_{\max.} = \sum \epsilon_{\lambda} L_{\lambda} \quad (7)$$

Using equation 7 in equation 2, the lower limit for the half-life is then given by

$$(t_{1/2})_{\min.} = \frac{0.693}{(k_{pE})_{\max.}} \quad (8)$$

The molar absorptivity can be determined experimentally by the method outlined in Section II.A and the values of L_{λ} are given in Tables 3 to 6 as a function of latitude and season of the year. These data can then be used in equation 7 to calculate $(k_{pE})_{\max.}$. Finally, $(k_{pE})_{\max.}$ can then be substituted in equation 8 to calculate $(t_{1/2})_{\min.}$.

In a second-tier test method, an aqueous photolysis screening test has been developed to determine rate constants and half-lives in the presence of sunlight using equations 1, 2, 4, 5, and 6 [USEPA (1979), Mill et al. (1981, 1982a, 1982b), and Dulin and Mill (1982)]. The second-tier test method is divided into two phases. In phase one, the test chemical is photolyzed in sunlight in order to obtain an approximate rate constant, k_p^C . This method only gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis.

In phase two, a standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) is used to measure sunlight intensities incident on the sample during photolysis [Mill et al. (1982b) and

Dulin and Mill. (1982)]. The rate constant for this actinometer, k_p^a , can be adjusted to match the approximate rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the quantum yield of the actinometer, the rate constant can be adjusted according to the equation

$$\phi_E^a = 0.0169 [\text{PYR}] \quad , \quad (9)$$

where [PYR] is the molar concentration of pyridine for a p-nitroacetophenone (PNAP) concentration of $1.00 \times 10^{-5} \text{M}$. The quantum yield for the test chemical, ϕ_E^c , is given by

$$\phi_E^c = \frac{k_p^c}{k_p^a} \frac{\sum \epsilon_\lambda^a L_\lambda}{\sum \epsilon_\lambda^c L_\lambda} \phi_E^a \quad (10)$$

The quantum yield of the test chemical, ϕ_E^c , can be determined in the following way. By measuring the concentration of test chemical and actinometer (PNAP) as a function of time t in sunlight, the ratio of rate constants, (k_p^c/k_p^a) , can be determined using equation 5 (Section II.B.1.h). ϕ_E^a can be determined from equation 9 at the molar concentration of pyridine used in the standard actinometer. The term $\sum \epsilon_\lambda^a L_\lambda$ for the actinometer has been tabulated as a function of latitude and season of the year [Table 2]. The term $\sum \epsilon_\lambda^c L_\lambda$ for the test chemical can be obtained from the experimentally measured molar absorptivities (Section II.A) and the values of L_λ , listed in Tables 3 to 6, as a function of latitude and season of the year.

With the values of ϕ_E^C , ϵ_λ^C , and the appropriate L_λ values, k_{pE} for the test chemical can be calculated as a function of latitude and season of the year in the United States using equation 4. The corresponding half-life can be calculated using k_{pE} in equation 2.

D. Applicability and Specificity

This Test Guideline is applicable to all chemicals which have uv-visible absorption maxima in the range 290-800nm. Some chemicals have absorption maxima significantly below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, saturated alcohols, and saturated acids). This is a direct consequence of the Grotthus-Draper law of photochemistry. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals.

These test methods are only applicable to pure chemicals and not to the technical grade.

The first-tier screening test can be employed to estimate $(k_{pE})_{\max}$ and $(t_{1/2})_{\min}$. If these data indicate that aqueous photolysis is an important process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.), then it is recommended that the second-tier photolysis tests be carried out to determine environmentally

relevant rate constants and half-lives in sunlight. The data obtained from this test can be used to determine k_{pE} for the test chemical as a function of latitude and season of the year in the United States. These rate constants are in the form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

The second-tier screening test is applicable to the direct photolysis of chemicals in a homogeneous dilute solution with absorbance less than 0.05 in the reaction cell at all wavelengths greater than 290 nm and at shallow depths (less than 0.5m). These results are applicable to direct sunlight photolysis for water bodies and clear sky conditions. In addition, these experiments are limited to the direct photolysis of chemicals in air-saturated pure water.

This screening test has been designed to determine the molar absorptivity of a test chemical, ϵ_{λ}^C , and its quantum yield, ϕ_E^C . These parameters can be used to determine environmental rate constants at low absorbance and shallow depths in pure water as a function of latitude and season of the year. Tables of solar irradiance (Table 3-6) have been included in this Test Guideline to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp and Cline (1977) have written a computer program to calculate the rates of photolysis as a function of depth in water, as a function of the attenuation

coefficient of the water (α_λ) for natural water bodies, the average ozone layer thickness that pertains to the seasons and location of interest, and as a function of latitude and season of the year. The computer program is available on request. [R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.]

II. TEST PROCEDURES

A. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-Life in Sunlight

The uv-visible absorption spectra in aqueous solution can be determined by the methods described in Test Guideline CG-1050. It is recommended that the following additional procedures be followed:

(1) For chemicals which ionize or protonate (e.g., carboxylic acids, phenols, amines), carry out uv-visible absorption studies at pHs at least two orders of magnitude above the pK_a and at least two orders of magnitude below the pK_a . Prepare buffer solutions at 25°C using reagent grade chemicals and distilled water as follows:

pHs in the range 3-6*: NaH_2PO_4
 HCl

pHs in the range 6-8* KH_2PO_4
 NaOH

pHs in the range >8: Prepare buffers as
described in the
Handbook of Chemistry
and Physics.

Check the pH of all the buffer solutions with a pH meter at 25°C and adjust to the proper pH, if necessary. These buffer solutions can then be added to the test chemical solution until the desired pH is obtained. If these buffers are inadequate, then adjust the pH of the test chemical solution with 1M HCl or NaOH at 25°C.

(2) Measure the absorbance, A_λ , as a function of wavelength in the range 290-800nm in duplicate. If applicable, measure A_λ at each experimental pH. Record, in duplicate, the baseline when both the sample and reference cells are filled with blank solutions. These data will be used to calculate the molar absorptivities for the appropriate wavelength intervals and

*Use the minimum concentration of buffers to attain the desired pH.

wavelength centers, Table 1 (Section V.A.), where the test chemical absorbs light. The wavelength center is defined as the midpoint of the interval range.

B. Tier 2 Test: Aqueous Photolysis in Sunlight

1. Test Conditions

a. Special Laboratory Equipment

It is recommended that quartz tubes be used for the photolysis of chemicals with appreciable absorption at wavelengths below 340nm. Chemicals that absorb appreciably at wavelengths greater than 340nm may be tested in borosilicate tubes. Thin-walled borosilicate or quartz tubes are recommended. Disposable culture tubes (13 x 100mm) with Teflon-lined screw caps or quartz tubes with quartz or borosilicate screw caps, Teflon-lined, may be used as reaction vessels. Tubes of 11mm i.d. are recommended. For some chemicals, it may be difficult to determine the concentration of the test chemical in reaction tubes of small volume. For these chemicals, larger volume reaction tubes are recommended provided that the cell walls are thin and the pathlength of radiation through the vessel is less than 0.5 meter.

b. Purity of Water

Reagent grade water, e.g., water meeting ASTM Type II A standards, or an equivalent grade, is highly recommended to minimize biodegradation. ASTM Type II A water is described in

ASTM D 1193-77, "Standard Specification for Reagent Water". Air-saturated water can be easily prepared by allowing the water to equilibrate in a vessel plugged with sterile cotton.

c. Sterilization

It is extremely important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions and in carrying out all photolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable non-chemical method.

d. pH Effects

It is recommended that all photolysis experiments be carried out at pHs at least two orders of magnitude above the pK_a and at least two orders of magnitude below the pK_a for any chemical which ionizes or protonates (e.g., carboxylic acids, phenols, and amines). Buffers described in Section II.B.2.b. should be used.

e. Volatile Chemical Substances

• • • Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels. Disposable culture tubes with Teflon-lined screw caps or quartz tubes with quartz or borosilicate screw caps, Teflon-lined, are

recommended. In addition, the reaction vessels should be as completely filled as is possible to prevent volatilization to any air space.

f. Control Solution

It is extremely important to take certain precautions to prevent loss of chemical from the reaction vessels by processes other than photolysis. For example, biodegradation and volatilization can be eliminated or minimized by use of sterile conditions and minimal air space in sealed vessels. Hydrolysis is a process which cannot be minimized by such techniques. Thus, control vessels containing test substance which are not exposed to sunlight are required. In this way, the loss of test chemical for processes other than photolysis may be determined and eliminated. For simplicity, if the loss of chemical in the control is small, one can calculate a first-order loss, k_{loss} , and subtract it from $(k_p)_{\text{obs}}$. to give the corrected direct photolysis rate constant k_p . If hydrolysis is found to be significant, hydrolysis studies should be carried out first. [Test Guideline CG-5000].

g. Absorption Spectrum as a Criterion for
Performing the Aqueous Photolysis Test

This aqueous photolysis screening test is applicable to all chemicals which have uv-visible absorption maxima in the range 290-800nm. Some chemicals have absorption maxima significantly

below 290nm but have measureable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290nm. Photolysis experiments should be carried out for these chemicals. The absorption spectrum of the chemical in aqueous solution can be measured by Test Guideline CG-1050.

h. Sunlight Actinometer

In order to quantify the rate of photolysis more precisely, it is necessary to measure the sunlight intensity incident on the sample during photolysis. A standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) has been developed [Mill et al. (1981, 1982b); Dulin and Mill (1982)] to measure the sunlight intensity incident on the sample during photolysis and this actinometer has been incorporated in this Test Guideline. According to equation 4, the rate constant is a function of the quantum yield. Furthermore, the quantum yield can be adjusted by varying the molar concentration of the pyridine according to equation 9. Hence, by varying the pyridine concentration, the actinometer photolysis rate constant can be adjusted so that the half-life can range from several hours to several weeks. The initial concentration of PNAP is set at $1.00 \times 10^{-5}M$.

Using the test chemical photolysis rate constant, k_p^c , determined in Tier 2, Phase 1, and the variable $k_a^a (= \sum \epsilon_{\lambda}^a L_{\lambda})$, listed in Table 2, the molar concentration needed to adjust the rate of disappearance of PNAP in PNAP/PYR to match the rate of disappearance of the test chemical is given by

$$[\text{PYR}] = 26.9 \left(\frac{k_p^c}{k_a^a} \right) \quad (11)$$

Experiments are carried out by simultaneously photolyzing the test chemical and actinometer solutions. The concentration of test chemical and actinometer are measured periodically as a function of time. These data are then used to determine the ratio of the rate constants, k_p^c/k_p^a , using linear regression analysis on the following equation

$$\ln(C_o/C_t)^c = (k_p^c/k_p^a) \ln(C_o/C_t)^a \quad (12)$$

The slope of the best straight line is the ratio of the rate constants, k_p^c/k_p^a .

i. Solar Irradiance Data

In order to calculate the quantum yield of the test chemical, ϕ_E^c , and then calculate k_{pE}^c and $q_{1/2}^c$, it is necessary to use the solar irradiance parameter L_λ . L_λ values are proportional to the average light flux that is available to cause photolysis in the wavelength interval λ over a 24-hour day at a specific latitude and season date. The L_λ values are defined by the angle of declination of the sun at -20° for winter, -10° for fall, $+10^\circ$ for spring and $+20^\circ$ for summer. The actual dates for 1982 that correspond to these angles of declination are January 21, April 16, July 24, and October 20, for winter, spring, summer and fall, respectively [AENA (1982)]. The L_λ values for these season dates are listed in Tables 3 to 6 (Section V.A) as a function of

latitude and are applicable to clear sky conditions, water bodies, shallow depths, and for chemicals whose absorbance is less than 0.05 in pure water [Mill et al. (1982a)].

j. Geometry of the Reaction Vessel

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body while the method developed in this Test Guideline measures rate constants (k_p) in tubes (e.g., 13 x 100mm). However, rates in tubes are faster than in water bodies and it has been experimentally observed [Mill et al. (1982b)] that

$$k_p = 2.2k_{pE} \quad (13)$$

Because tubes are the simplest and easiest reaction vessels to use, this Test Guideline recommends the use of tubes as reaction vessels and the method has been modified to take into account the increased rate in tubes (equation 13).

k. Chemical Analysis of Solutions

In determining the concentration of the chemical in solution, an analytical method should be selected which is most applicable to the analysis of the specific chemical substance. Chromatographic methods are generally recommended because of their chemical specificity in analyzing the parent chemical

substance without interference from impurities. Whenever practicable the chosen analytical method should have a precision of ± 5 percent or better.

The p-nitroacetophenone in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30cm C₁₈ reverse-phase column and a uv detector set at 280nm. The mobile phase in volume percent is 2.5% acetic acid, 50% acetonitrile, and 47.5% water which is passed through the column at a flow rate of 2 mL/minute.

2. Preparations

a. Preparation of Test Chemical Solution

Prepare homogeneous solutions with the chemical at less than one-half its solubility in water and at a concentration such that the absorbance is less than 0.05 in the photolysis reaction vessel at wavelengths greater than 290nm. For very hydrophobic chemicals, it is difficult and time consuming to prepare aqueous solutions. To facilitate the preparation of aqueous solutions containing very hydrophobic chemicals and to allow for easier analytical measurement procedures, the following procedure may be used to aid in the dissolution of the chemical. Dissolve the pure chemical in reagent grade acetonitrile. Add pure water as described under Test Conditions, Section II.B.1.b, or buffer solution as described under Preparations, Section II.B.2.b., for chemical substances which ionize or protonate, to an aliquot of

the acetonitrile solution. Do not exceed one volume-percent of acetonitrile in the final solution. Place the reaction solution in the appropriate photolysis reaction tubes as described in Section II.B.1.a.

b. Preparation of Buffer Solutions

Prepare buffer solutions according to the procedures outlined in Section II.A.1 using reagent grade chemicals and pure water as described under Test Conditions, Section II.B.1.b.

c. Preparation of Actinometer Solution

Using the test chemical photolysis rate constant, k_p^C , determined in Tier 2, Phase 1, and the variable k_a^a listed in Table 2, the molar concentration of pyridine needed to adjust the rate of disappearance of p-nitroacetophenone (PNAP) to match the rate of disappearance of the test chemical can be obtained from equation 11. The variable $k_a^a (= \int \epsilon_\lambda L_\lambda)$ is equal to the day-average rate constant for sunlight absorption by PNAP which changes with season and latitude. The value of k_a^a is selected from Table 2 for the season nearest the mid-experiment date of the Tier 2, Phase 1, studies and the decadic latitude nearest the latitude of the experimental site.

Once the molar concentration of pyridine [PYR] has been determined, an actinometer solution can be prepared as follows. Dissolve 0.165 of PNAP in 100 mL of acetonitrile (0.01M). Add 1

mL of this solution to a one liter volumetric flask. Add to the volumetric flask the mass in grams, or the volume (V) of pyridine at 20°C, obtained from the equations

$$\text{mass(grams)} = 79.1[\text{PYR}]$$

$$V(\text{mL}) = 80.6[\text{PYR}] \quad . \quad (14)$$

Fill the volumetric flask with pure water (Section II.B.1.b.) to give one liter of solution and shake vigorously to make sure that the solution is homogeneous. The PNAP/PYR solution should be wrapped with aluminum foil and kept from bright light.

3. Performance of the Tests

a. Phase 1 Experiments

For all experiments, prepare an aqueous solution of the chemical substance, as described in Section II.B.2.a., and a sufficient number of samples in quartz or borosilicate glass tubes to perform all the required tests. Fill the tubes as completely as possible and seal them. Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminum foil or by other suitable methods. These samples are analyzed for the chemical substance immediately after completion of the experiment to measure the loss of chemical in the absence of light. Place the samples, including the controls, outdoors in an area free of

shade and reflections of sunlight from windows and buildings. Place the samples on a black, non-reflective background and inclined at approximately 30° from the horizontal with the upper end pointing due north (in the northern hemisphere). Conduct the photolysis experiments during a frost-free time of year (e.g., May, June, July, August, or September in the northern hemisphere-weather permitting) and start the experiments initially at noon (1200 hours). Record the date and time the experiment was begun, the date and time completed, the time of sunrise and sunset on all days when photolysis experiments were performed, the times exposure was stopped and restarted for intermittent exposure, the weather conditions during the period, and the latitude of the site. For chemical substances that ionize or protonate, carry out photolysis experiments at the required pHs as described under Test Conditions, Section II.B.1.d.

If a significant loss of test chemical has occurred in the control samples, determine the cause and eliminate or minimize the loss. If hydrolysis is found to be significant, hydrolysis studies should be carried out first (Section II.B.1.f.).

Use one of the following procedures, depending on how fast the chemical substance photolyzes.

i. Procedure 1

If the chemical substance transforms 50-80% within 28 days, measure the concentration of the chemical substance, in duplicate, at time $t = 0$ and periodically (at least four data

points at approximately equal time intervals) at noon (1200 hours) until at least 50% of the substance has been consumed. As a simplification, the sampling times can be estimated as the photolysis experiments are in progress. Determine the concentration of test chemical from two, freshly opened, reaction tubes for each time point. Determine the concentration in each of the two control solutions as soon as the photolysis experiments are completed.

ii. Procedure 2

If the chemical substance transforms in the range of 20-50% in 28 days, determine the concentration of the chemical substance, in duplicate, at time $t = 0$. Determine the concentration of the chemical in the two separate reaction tubes and the two control tubes after 28 days of photolysis.

iii. Procedure 3

For chemical substances that transform in sunlight 50-80% within one or two days, place the samples outside at noon (1200 hours) and analyze two samples for the concentration of the chemical substance at $t = 0$, and in two, freshly opened, reaction tubes at noon (1200 hours) the next day, and again, in two, freshly opened, reaction tubes at noon (1200 hours) the second day. Determine the concentration of the test chemical in each of the two control solutions as soon as the photolysis experiments have been completed.

iv. Analytical Methodology

Select an analytical method which is most applicable to the analysis of the specific chemical being tested (Section II.B.1.k.).

b. Phase 2 Experiments

Using the test chemical photolysis rate constant, k_p^c , determined in Tier 2, Phase 1, prepare an actinometer solution, as described in Section II.B.2.c., and a sufficient number of samples in quartz tubes to perform all the required tests. Fill all the tubes as completely as possible, seal them, and cover them with aluminum foil as soon as possible after preparation. Prepare an aqueous solution of test chemical, as described in Section II.B.2.a, and a sufficient number of samples in quartz or borosilicate tubes to perform all the required tests. Fill these tubes as completely as possible, seal them, and cover them with aluminum foil as soon as possible after preparation. Place all the samples outdoors in an area free of shade and reflections of sunlight from windows and buildings. Place the samples on a black, nonreflective background and inclined at approximately 30° from the horizontal with the upper end pointing due north (in the northern hemisphere). Remove the foil from all samples except for the test chemical control solutions and the actinometer control solutions at noon (1200 hours). Based on the results of the Phase 1 experiments, determine the concentration of test chemical and actinometer (PNAP), in triplicate, at time $t = 0$ and

periodically (at least five data points at approximately equal time intervals). Determine the concentration of PNAP in the three actinometer control solutions and the concentration of test chemical in the three control solutions for each time point.

Select an analytical method which is most applicable to the analysis of the specific chemical tested, Section II.B.1.k., and follow the procedure given in Section II.B.1.k. for the analysis of PNAP.

III. DATA AND REPORTING

A. Tier 1 Test: UV-Visible Absorption Spectra - Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-life in Sunlight

1. Treatment of Results

The molar absorptivity can be determined from the absorption spectra using the expression

$$\epsilon_{\lambda} = \frac{A_{\lambda}}{C \ell} \quad (15)$$

where A_{λ} is the absorbance at wavelength λ , C is the molar concentration of test chemical, and ℓ is the cell pathlength in centimeters. The molar absorptivity of the chemical should be determined for the wavelengths listed in Table 1 for a solution

of concentration C and in a cell with pathlength l . If the absorption curve is flat within the interval around the wavelength λ_{center} , ϵ_{λ} may be determined from the absorbance A_{λ} at λ_{center} using equation 15. If a large change in absorbance occurs within this interval, obtain an average absorbance A_{λ} at λ_{center} based on the two absorbances at the boundary of the interval. Calculate an average ϵ_{λ} using the average value of A_{λ} in equation 15. Determine the molar absorptivity for each replicate and calculate a mean value.

Using the molar absorptivities obtained from the spectra and the values of the L_{λ} from Tables 3 to 6, the maximum rate constant $(k_{pE})_{\text{max}}$ can be calculated at a specific latitude and season of the year using equation 7. The minimum half-life, $(t_{1/2})_{\text{min}}$ can then be calculated using this $(k_{pE})_{\text{max}}$ in equation 8.

Two hypothetical examples are presented in an Appendix, Section V.B.1, to illustrate how the test data obtained in the first-tier screening test can be used.

2. Test Data Report

- (1) Submit the original chart, or photocopy, containing a plot of absorbance of test chemical vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10nm intervals. Each spectrum should be clearly marked with the test conditions.

- (2) Report the concentration of the test chemical solution, the type of absorption cell used (quartz or borosilicate glass) and the pathlength.
- (3) Report A_{λ} and ϵ_{λ} at λ_{center} for each replicate and the mean value.
- (4) Report the identity or composition of the solvent used in the spectral absorption study.
- (5) For ionizable chemicals, report its pKa. Report the type and concentration of the buffers employed for each pH.
- (6) Describe the method employed in determining the test chemical's concentration.
- (7) Report the name, structure, and purity of the test chemical.
- (8) Submit a recent test spectrum on appropriate reference chemicals for photometric and wavelength accuracy.
- (9) Report the name and model of the spectrophotometer used.
- (10) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, gain, etc.

B. Tier 2 Test: Aqueous Photolysis in Sunlight

1. Phase 1 Experiments

a. Treatment of Results

If a small loss of test substance in the control tubes has occurred, use this data to make corrections to the measured photolysis rate (Section II.B.1.f.). Note the site of photolysis and its latitude and the weather conditions. For procedures 1 and 2 note the dates and times of actual exposure including times of sunrise and sunset and, in case the cells are moved to prevent freezing or for other reasons, make sure that these times are recorded and that the cells are kept in a dark place when exposure is not in progress.

- (1) For chemical substances which transform 50-80% within 28 days, use a concentration C , which corresponds to less than 50% of the initial concentration of chemical substance remaining, and the corresponding time t , in days, along with the initial molar concentration C_0 , in equation 5 to calculate k_p in days^{-1} . From the analysis of the two samples at time $t = 0$ and t , calculate a mean value of C_0 and C , respectively, and a value of k_p . If a slight loss of chemical has been detected in the controls, then calculate a rate constant as follows: Calculate an average concentration C , based on the duplicate measurements of

concentration in the controls. Use this concentration along with the average initial concentration in equation 5 and calculate a rate constant k_{loss} . Using this rate constant along with the observed rate constant, the corrected rate constant is then

$$k_p = (k_p)_{obs.} - k_{loss} \quad (16)$$

Calculate the half-life, $t_{1/2}$ using the corrected k_p value in equation 6;

- (2) for chemical substances which transform 20-50% in 28 days, use the mean concentration C remaining at $t = 28$ days along with the mean value of C_0 to calculate k_p . Use the same procedure as described above to calculate the value of k_p and $t_{1/2}$. If less than 20% of the chemical substance degrades in 28 days, report the mean concentration of C and C_0 . In this case the apparent half-life is reported as greater than 3 months; and
- (3) for chemical substances which transform 50% or more in the first day, as described in procedure 3, calculate a full day k_p value using the mean concentration C of chemical substance remaining at noon (1200 hours) after the first day along with the mean value of C_0 using equation 5. For chemical substances which degrade less than 50% at noon (1200 hours) after the first day but 50% or more at noon (1200 hours) the second day,

calculate k_p using the mean concentration of chemical substances remaining at noon (1200 hours) the second day. Calculate the half-life, $t_{1/2}$ using the mean value of k_p in Equation 6. If a small loss of test substance in the control tubes has occurred, use this data to make corrections to the measured photolysis rate as described above. Note the dates of photolysis, the latitude, and the site.

A hypothetical example is presented in an Appendix, Section V.B.2, to illustrate how the test data obtained in the Tier 2, Phase 1, test method can be used.

b. Specific Analytical and Recovery Procedures

- (1) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision; and
- (2) if extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

c. Other Test Conditions

- (1) Report the size, approximate cell wall thickness, and type of glass used for the reaction tubes;
- (2) report the initial pH of all test solutions, if appropriate;

- (3) for all procedures, report the dates of photolysis, the time of sunrise and sunset on each photolysis day, the site of photolysis and its latitude, and the weather conditions. For procedures 1 and 2 submit the dates and times of actual exposure, and the duration of exposure, and, for intermittent exposure, the fraction of each day during which photolysis occurred;
- (4) if acetonitrile was used to solubilize the test substance, report the percent, by volume; and
- (5) if a significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

d. Test Data Report

- (1) For each photolysis experiment, report:
 - i. The initial molar concentration of test chemical (C_0) of each replicate and the mean value.
 - ii. The molar concentration of test chemical for each replicate and the mean value for each time point t .
 - iii. The molar concentration of each replicate control sample and the mean value after completion of the photolysis experiments.
- (2) For procedure 1, 2, or 3, report the value of k_p . If small losses of chemical are observed, report $(k_p)_{obs.}$, k_{loss} and k_p . Report the half-life ($t_{1/2}$) calculated using the value of k_p .

2. Phase 2 Experiments

a. Treatment of Results

The objective of this set of experiments is to determine the environmental quantum yield, ϕ_E^C , for a specific test chemical.

ϕ_E^C can be calculated using equation 10,

$$\phi_E^C = \frac{k_p^C}{k_p^a} \frac{\sum \epsilon_{\lambda}^a L_{\lambda}}{\sum \epsilon_{\lambda}^C L_{\lambda}} \phi_E^a, \quad (10)$$

by the following steps:

- (1) Determine the ratio of the rate constants, k_p^a/k_p^C , as described in Section II.B.1.h using equation 12. If a slight loss of test chemical or actinometer (PNAP) was detected in the controls at any time t, then employ the following procedure. Consider, as an example, the loss of test chemical in the control at time t. Using the average concentration of the test chemical in the controls from the replicates at time t and the average initial concentration, calculate $\ln(C_o/C_t)^C_{\text{loss}}$. Using the average concentration of test chemical from the replicates after photolysis time t, calculate $\ln(C_o/C_t)^C_{\text{obs}}$. The corrected term is then

$$\ln(C_o/C_t)^C_{\text{corr.}} = \ln(C_o/C_t)^C_{\text{obs.}} - \ln(C_o/C_t)^C_{\text{loss.}} \quad (17)$$

The same procedure can be applied to obtain a corrected term from the actinometer (PNAP).

Using the corrected terms for test chemical and/or actinometer in equation 12, determine the ratio of the rate constants (k_p^a/k_p^c) as described in Section II.B.1.h.

- (2) Determine the quantum yield of the actinometer, ϕ_E^a , using equation 9 and the molar concentration of pyridine [PYR] present in the actinometer.
- (3) Determine the value of $\sum \epsilon_\lambda^c L_\lambda$, for the test chemical, as follows: the molar absorptivities, ϵ_λ^c , have been determined by the procedure given in Section II.A. and the results have been tabulated according to Section III.A.2. Choose the appropriate L_λ values (Tables 3 to 6) that correspond to the season closest to the season in which the Phase 2 experiments were performed and to the latitude nearest the latitude of the experimental site. Calculate the product of ϵ_λ^c and L_λ for each wavelength interval where ϵ_λ has a nonzero value. Sum the products of $\epsilon_\lambda L_\lambda$ over all wavelength intervals.
- (4) Determine the value of $\sum \epsilon_\lambda^a L_\lambda$, for the actinometer, as follows: These values have been calculated and are given in Table 2. Choose the appropriate value that corresponds to the season closest to the season in which the Phase 2 experiments were performed and to the latitude nearest the latitude of the experimental site.

- (5) Substitute the values of k_p^a/k_p^c , ϕ_E^a , $\sum \epsilon_\lambda^a L_\lambda$, and $\sum \epsilon_\lambda^c L_\lambda$ in equation 10 and calculate ϕ_E^c , the quantum yield of the test chemical in the environment (i.e., in sunlight).

Once ϕ_E^c has been determined, equation 4 can be used to calculate k_{pE} at any season of the year and latitude using the measured values of the molar absorptivities, ϵ_λ^c , and the appropriate L_λ values (Tables 3 to 6). The half-life can then be calculated using k_{pE} in equation 2.

A hypothetical example is presented in an Appendix, Section V.B.3, to illustrate how the test data is obtained in the Tier 2, Phase 1, test method can be used.

b. Other Test Conditions

- (1) Report the size, approximate cell wall thickness, and type of glass used for tubes used to hold the test chemical and actinometer solutions.
- (2) Report the initial pH of all test chemical solutions, if appropriate, and the type and concentration of the buffers employed for each pH.
- (3) If acetonitrile was used to solubilize the test chemical, report the percent, by volume, of the acetonitrile, which was used.

- (4) If significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

c. Test Data Report

- (1) Report the initial molar concentration of chemical (C_0) of each replicate and the mean value.
- (2) Report the initial molar concentration of PNAP and the molar concentration of pyridine used in the actinometer.
- (3) Report the time and date the sunlight photolysis experiments were started, the time and date the experiments were completed, and the elapsed photolysis time in days.
- (4) For each time point, report the three separate values for the molar concentration of test chemical and PNAP and the mean values.
- (5) For each time point, report the three separate values of the molar concentration of test chemical and PNAP for the controls and the mean values.
- (6) Tabulate and report the following data: t , $\ln(C_0/C_t)^C$, and $\ln(C_0/C_t)^A$. From the linear regression analysis, report the ratio of the rate constants, k_p^C/k_p^A , and the correlation coefficient.
- (7) If loss of test chemical and/or actinometer was observed during photolysis, then report the data $\ln(C_0/C_t)_{\text{corr.}}$, $\ln(C_0/C_t)_{\text{obs.}}$, $\ln(C_0/C_t)_{\text{loss}}$ for the

- test chemical and/or actinometer at each time t . From the linear regression analysis of $\ln(C_o/C_t)^C_{\text{corr.}}$ and $\ln(C_o/C_t)^a_{\text{corr.}}$, report the ratio of the rate constants, k_p^C/k_p^a and the correlation coefficient.
- (8) Report the quantum yield of the actinometer (ϕ_E^a).
 - (9) Report the value of k_a^a for the actinometer corresponding to the season closest to the season in which the photolysis experiments were carried out and to the latitude nearest the latitude of the experimental site.
 - (10) Tabulate the values of λ_{center} , ϵ_{λ}^C , L_{λ} , and $\epsilon_{\lambda}^C L_{\lambda}$ for the test chemical corresponding to the season closest to the season in which the photolysis experiments were carried out and to the latitude nearest the latitude of the experimental site.
 - (11) Report the value $\int \epsilon_{\lambda}^C L_{\lambda}$ for the test chemical from step 10.
 - (12) Report the quantum yield of the test chemical.
 - (13) For chemicals that ionize, report the data for steps 1-12 for the experiments at the required pHs.

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V. APPENDICESA. Appendix A. Tables 1 - 6Table 1. WAVELENGTH CENTER AND INTERVALS FOR L_{λ}
(in nm)

λ Center ¹	Interval Range		$\Delta\lambda$
	From	To	
299	296.3	301.3	5.0
304	301.3	306.3	5.0
309	306.3	311.3	5.0
314	311.3	316.3	5.0
319	316.3	321.3	5.0
323	321.3	325.0	3.7
340	325.0	355.0	30.0
370	355.0	385.0	30.0
400	385.0	415.0	30.0
430	415.0	445.0	30.0
460	445.0	475.0	30.0
494	475.0	521.5	37.5
537	512.5	562.5	50.0
588	562.5	612.5	50.0
638	612.5	662.5	50.0
691	662.5	718.8	56.3
747	718.8	775	56.2
800	775	825	50.0

¹Rounded off to unit wavelength.

Table 2. DAY AVERAGED RATE CONSTANT (k_a^a)* FOR SUNLIGHT ABSORPTION BY PNAP AS
A FUNCTION OF SEASON AND DECADIC LATITUDE

Latitude	Season			
	Spring	Summer	Fall	Winter
20°N	389	422	321	260
30°N	371	426	265	189
40°N	330	410	235	99
50°N	279	387	132	60

$$*k_a^a = \int \epsilon_\lambda^a L_\lambda \text{ in day}^{-1}$$

TABLE 3. L_λ VALUES FOR LATITUDE 20°N^{†,a}

λ Center	Spring	Summer	Fall	Winter
299	3.51(-4)	4.44(-4)	2.74(-4)	1.47(-4)
304	2.51(-3)	3.15(-3)	2.20(-3)	1.47(-3)
309	8.09(-3)	9.61(-3)	6.89(-3)	5.34(-3)
314	1.81(-2)	1.97(-2)	1.48(-2)	1.15(-2)
319	2.82(-2)	3.02(-2)	2.33(-2)	1.88(-2)
323	2.83(-2)	3.03(-2)	2.33(-2)	1.88(-2)
340	3.29(-1)	3.47(-1)	2.68(-1)	2.21(-1)
370	4.24(-1)	4.47(-1)	3.45(-1)	2.86(-1)
400	8.41(-1)	8.83(-1)	6.96(-1)	5.97(-1)
430	1.17	1.23	9.80(-1)	8.40(-1)
460	1.47	1.55	1.24	1.06
494	1.88	1.98	1.58	1.36
537	2.68	2.81	2.30	1.95
588	2.80	2.96	2.35	2.03
638	2.80	2.90	2.42	2.07
691	3.15	3.38	2.70	2.36
747	3.10	3.15	2.47	2.65
800	2.50	2.70	2.26	1.60

[†]. Units of L_λ are 2.303×10^{-3} einsteins $\text{cm}^{-2} \text{day}^{-1}$. Multiplication of L_λ by ϵ_λ in units of $\text{molar}^{-1} \text{cm}^{-1}$ gives rate constant in units of day^{-1} .

^a. The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

TABLE 4. L_λ VALUES FOR LATITUDE 30°N

λ Center	Spring	Summer	Fall	Winter
299	2.30(-4)	3.65(-4)	1.35(-4)	4.10(-5)
304	2.13(-3)	2.32(-3)	1.44(-3)	6.50(-4)
309	7.26(-3)	9.02(-3)	4.84(-3)	2.76(-3)
314	1.65(-2)	1.92(-2)	1.16(-2)	7.55(-3)
319	2.64(-2)	3.02(-2)	1.89(-2)	1.31(-2)
323	2.69(-2)	3.04(-2)	2.30(-2)	1.34(-2)
340	3.20(-1)	3.74(-1)	2.23(-1)	1.70(-1)
370	4.14(-1)	4.37(-1)	2.84(-1)	2.19(-1)
400	8.27(-1)	9.07(-1)	6.23(-1)	4.75(-1)
430	1.15	1.34	8.50(-1)	6.69(-1)
460	1.45	1.59	1.09	8.50(-1)
494	1.85	2.03	1.39	1.10
537	2.64	2.89	2.00	1.57
588	2.74	3.03	2.07	1.63
638	2.76	3.00	2.09	1.67
691	3.15	3.38	2.36	1.95
747	3.04	3.26	2.36	1.83
800	2.50	2.80	1.90	1.60

TABLE 5. L_{λ} VALUES FOR LATITUDE 40°N

λ Center	Spring	Summer	Fall	Winter
299	1.09(-4)	2.49(-4)	1.09(-4)	5.38(-6)
304	1.37(-3)	2.32(-3)	1.37(-3)	1.56(-4)
309	5.35(-3)	7.93(-3)	5.35(-3)	1.02(-3)
314	1.38(-2)	1.81(-2)	1.38(-2)	3.79(-3)
319	2.31(-2)	2.91(-2)	2.31(-2)	7.53(-3)
323	2.59(-2)	2.97(-2)	2.39(-2)	8.10(-3)
340	2.98(-1)	3.54(-1)	1.08(-1)	7.52(-2)
370	3.89(-1)	4.58(-1)	3.84(-1)	1.47(-1)
400	7.91(-1)	9.71(-1)	7.91(-1)	3.38(-1)
430	1.11	1.28	1.11	4.80(-1)
460	1.39	1.43	1.39	6.10(-1)
494	1.42	2.04	1.78	7.75(-1)
537	2.52	2.92	1.69	1.12
588	2.62	3.05	1.73	1.16
638	2.60	3.00	1.78	1.19
691	1.69	3.49	1.50	1.56
747	1.91	3.26	1.6	1.35
800	1.61	2.90	1.6	1.16

TABLE 6. L_{λ} VALUE FOR LATITUDE 50°N

λ Center	Spring	Summer	Fall	Winter
299	3.71(-5)	7.88(-6)	1.52(-4)	4.00(-7)
304	7.10(-4)	1.75(-3)	2.25(-4)	1.57(-5)
309	3.55(-3)	6.53(-3)	1.29(-3)	1.78(-4)
314	7.30(-3)	1.63(-2)	4.39(-3)	1.20(-3)
319	1.84(-2)	2.67(-2)	8.64(-3)	2.93(-3)
323	1.96(-2)	2.77(-2)	9.20(-3)	3.68(-3)
340	2.66(-1)	3.43(-1)	1.24(-1)	6.29(-2)
370	3.48(-1)	4.44(-1)	1.66(-1)	8.21(-2)
400	7.24(-1)	9.04(-1)	3.65(-1)	1.96(-1)
430	1.02	1.26	5.17(-1)	2.75(-1)
460	1.29	1.60	6.60(-1)	3.51(-1)
494	1.65	2.04	8.50(-1)	4.44(-1)
537	2.34	2.90	1.22	6.30(-1)
588	2.40	3.04	1.25	6.40(-1)
638	2.44	3.00	1.31	6.90(-1)
691	2.82	3.49	1.51	7.99(-1)
747	2.81	3.26	1.47	7.98(-1)
800	2.30	2.90	1.24	6.90(-1)

B. Appendix B. Illustrative Examples1. Tier 1 Test: UV-Visible Absorption Spectra -
Estimation of Aqueous Photolysis Rate Constant and
Minimum Half-life in Sunlight

a. Illustrative Example 1

A neutral organic chemical A was dissolved in water at a concentration of $1.00 \times 10^{-3}M$. UV-visible absorption spectra were obtained in a 10cm quartz absorption cell and no absorbance was detected above the baseline in the region 290nm and greater (i.e., $A_\lambda = 0$ for $\lambda > 290nm$). Since $A_\lambda = 0$, then $\epsilon_\lambda = 0$ (equation 15). Using this result in equation 7, it is found that $(k_{pE})_{max.} = 0$, indicating that no direct photolysis can take place in sunlight at any latitude or season of the year. This example illustrates the principle of the Grotthus-Draper law, the first law of photochemistry. That is, in order for direct photolysis to take place in sunlight, the chemical must absorb sunlight in the region $\lambda > 290nm$.

b. Illustrative Example 2

Consider a plant located in Columbus, Georgia on the Chattahoochee River which produces an organic chemical B which is not an acid or a base. The waste effluent passes through a primary and secondary treatment plant and is then discharged directly into the river. The plant produces chemical B

continuously every day of the year. The plant is located at 32.5° north latitude. Estimate the maximum sunlight direct photolysis rate constant and the corresponding minimum half-life for this chemical in the river for the winter and summer seasons under clear skies.

Laboratory Experiments, Data, and Calculations

The water solubility of chemical B is $1.00 \times 10^{-3} \text{M}$ at 25°C. Chemical B was dissolved directly in water and a 1.00×10^{-4} molar solution was prepared at 25°C. The uv-visible absorption spectra were obtained according to the Tier 1 Procedure in a 10.0cm quartz absorption cell in duplicate. Using the wavelength interval range (from Table 1), the average absorbance of the duplicate runs at λ_{center} was obtained and the results are summarized in Table 7.

Table 7. SUMMARY OF PHOTOLYSIS DATA FOR CHEMICAL B

λ center (nm)	A_λ	ϵ_λ (M ⁻¹ cm ⁻¹)
299	1.534	1534
304	1.000	1000
309	0.200	200
314	0.100	100
319	0.010	10
323	0.001	1
340	0.000	0

λ center (nm)	Summer		Winter	
	L_λ^*	$\epsilon_\lambda L_\lambda$ (day ⁻¹)	L_λ^*	$\epsilon_\lambda L_\lambda$ (day ⁻¹)
299	3.65(-4)	0.56	4.10(-5)	0.06
304	2.32(-3)	2.32	6.50(-4)	0.65
309	9.02(-3)	1.80	2.76(-3)	0.55
314	1.92(-2)	1.92	7.55(-3)	0.76
319	3.02(-2)	0.30	1.31(-2)	0.13
323	3.04(-2)	0.03	1.34(-2)	0.01
340	3.74(-1)	0.00	1.70(-1)	0.00

$$\sum \epsilon_\lambda L_\lambda = 6.93$$

$$\sum \epsilon_\lambda L_\lambda = 2.16$$

* The units of L_λ are in 2.303×10^{-3} einsteins cm⁻² day⁻¹.

From the above data and equation 15, the average molar absorptivity is

$$\epsilon_\lambda = 1000 A_\lambda \quad (18)$$

From the average A_λ value at λ_{center} , the average molar absorptivity can be obtained from equation 18 and the results are summarized in Table 7. Since the plant is located at 32.5° north latitude, the closest L_λ values are at 30° north latitude. These values are obtained from Table 4 and are summarized in Table 7 for the summer and winter seasons. Using the data from Table 7 and equations 7 and 8, the following results are obtained.

<u>Summer</u>	<u>Winter</u>
$(k_{pE})_{\text{max.}} = \sum \epsilon_\lambda L_\lambda = 6.93 \text{ day}^{-1}$	$(k_{pE})_{\text{max.}} = \sum \epsilon_\lambda L_\lambda = 2.16 \text{ day}^{-1}$
$(t_{1/2})_{\text{min.}} = 0.10 \text{ day}$	$(t_{1/2})_{\text{min.}} = 0.32 \text{ day}$

Since the chemical transforms rapidly on the summer and winter season dates for 1982, it is necessary to carry out Tier 2 experiments to more accurately define direct photolysis rates in aqueous media as a function of the season of the year.

2. Tier 2, Phase 1: Aqueous Photolysis in Sunlight

a. Illustrative Example 3

Consider the same scenario as described in illustrative example 2, Section V.B.1.b. Using the Tier 2, Phase 1 Procedure, carry out experiments to estimate the rate of direct photolysis and half-life in aqueous solution in the spring for water bodies.

Photolysis Experiments and Calculations

Since chemical B absorbs appreciably below 340nm, 11mm i.d. quartz tubes were used (note: this tube has an approximate pathlength of 1cm). Chemical B was dissolved directly in pure water and a 1.00×10^{-5} molar solution was prepared at 25°C. Since the water solubility is 1.00×10^{-3} M at 25°C, this sample solution was well below one-half its water solubility. The uv spectrum of this solution in a one cm absorption cell indicated that A_{λ} was less than 0.05 at $\lambda > 290\text{nm}$. Hence, under these conditions, first-order kinetics are applicable.

A series of quartz tubes were filled with this aqueous solution, sealed, and photolysis experiments were carried out in sunlight according to the appropriate procedure described in Section II.B.3.a. The experiments were started at noon (1200 hours) on May 8, 1982. The weather conditions are summarized for this period of time and the concentration data given represent the mean of duplicate determinations.

- (1) May 2, 1982: at $t = 0$ (noon = 1200 hours)
 $C_0 = 1.00 \times 10^{-5}\text{M}$.
- (2) May 2, 1982: Noon to sunset - clear and sunny.
- (3) May 3, 1982: Noon (1200 hours), $C = 0.840 \times 10^{-5}\text{M}$.
- (4) May 3, 1982: at 1400 hours the weather conditions were cloudy with rain. The rain and cloudy weather continued until 2200 hours.

- (5) From sunrise, May 4, 1982 through 1200 hours May 8, 1982, the weather was clear and sunny. At 1200 hours, May 8, 1982, analysis of the samples gave an average concentration of $C = 0.400 \times 10^{-5}M$. Since 60% of chemical B transformed, the photolysis experiments were terminated and the control samples were analyzed. The average concentration of the control samples was $0.997 \times 10^{-5}M$ which was essentially the same as C_0 . Hence, no adventitious processes occurred and the loss of chemical was only due to sunlight photolysis.

Listed in Table 8 are the times of sunrise and sunset for the dates sunlight photolysis experiments were carried out along with the total number of hours of sunlight.

Table 8. SUMMARY OF TIMES FOR SUNRISE AND SUNSET FOR THE PERIOD MAY 2-8, 1982

<u>Date (1982)</u>	<u>Sunrise (AM)</u>	<u>Sunset (PM)</u>	<u>Total Sunlight Hours</u>
5/2	0600	2010	14.2
5/3	0559	2011	14.2
5/4	0558	2012	14.2
5/5	0557	2013	14.3
5/6	0556	2014	14.3
5/7	0555	2015	14.3
5/8	0554	2016	14.4

The following data summarizes the dates photolyzed, the times exposed to sunlight, the total sunlight photolysis time for each date in days, and the total number of days of sunlight photolysis.

Date	Times Photolyzed	Sunlight Photolysis Time for Each Date (days)
5/2	1200 hrs. to 2010 hrs. (8.2/14.2)	0.58
5/3	0559 hrs. to 1200 hrs. (6.0/14.2)	0.42
5/3	1200 hrs. to 1400 hrs. (2.0/14.2)	0.14
5/4	0558 hrs. to 2012 hrs.	1.00
5/5	0557 hrs. to 2013 hrs.	1.00
5/6	0556 hrs. to 2014 hrs.	1.00
5/7	0555 hrs. to 2015 hrs.	1.00
5/8	0554 hrs. to 1200 hrs. (6.1/14.4)	0.42
		total 5.56 days

$$t = 5.6 \text{ days}; C_0 = 1.00 \times 10^{-5}; C = 0.400 \times 10^{-5}$$

$$\ln \left(\frac{C_0}{C} \right) = k_p t$$

$$k_{pE} = \frac{1}{t} \ln \left(\frac{C_0}{C} \right) = \frac{1}{5.6 \text{ days}} \ln \left(\frac{1.00 \times 10^{-5}}{0.400 \times 10^{-5}} \right)$$

$$k_p = 0.16 \text{ days}^{-1}$$

$$t_{1/2} = \frac{0.693}{0.16 \text{ days}^{-1}} = 4.3 \text{ days}$$

Therefore, the rate constant for direct photolysis of chemical B in tubes in pure water is 0.16 days^{-1} and the corresponding half-life is 4.3 days for the period of photolysis May 2-8, 1982 at 32.5° north latitude. Using equation 11, the direct photolysis rate constant (k_{pE}) for water bodies is 0.073 days^{-1} and the corresponding half-life ($t_{1/2}$) is 9.5 days.

3. Tier 2, Phase 2: Aqueous Photolysis in Sunlight

i. Illustrative Example 4

Consider the same scenario as described in illustrative examples 2 and 3. Using the Tier 2, Phase 2, Procedure, carry out experiments to determine the sunlight quantum yield and estimate the rate constant for direct photolysis in aqueous solution and the half-life for water bodies and clear sky conditions for the summer and winter seasons.

Photolysis Experiments and Calculations

The sunlight photolysis experiments were carried out in the beginning of May, 1982 at 32.5° north latitude.

(1) Preparation of the Actinometer Solution

The results from the Tier 2, Phase 1, experiments indicated that k_p^C for the test chemical was 0.16 days⁻¹. Since the experiments were carried out in early May at 32.5° north latitude, the value of k_a^a was chosen from Table 2 which corresponds to the spring season and at 30° north latitude. Using equation 11, the pyridine required to adjust the actinometer rate to match the rate of disappearance of the test chemical is

$$[\text{PYR}] = 26.9 \left(\frac{0.16}{371} \right) = 0.0116 \text{ molar}$$

Using the concentration of pyridine, an actinometer solution was prepared according to the procedure described in Section II.B.h. The quantum yield for this actinometer is calculated using equation 9.

$$\phi_E^a = 0.0169[\text{PYR}] = 0.0169(0.0116) = 1.96 \times 10^{-4}$$

Procedures for Tier 2, Phase 2 experiments (Section II.B.3.b) were followed and sunlight experiments were initiated at 1200 hours on May 9, 1982. The mean initial concentration of test chemical was 1.00×10^{-5} molar and the mean initial concentration of PNAP was 1.00×10^{-5} molar. Samples of the chemical and actinometer were analyzed in triplicate periodically at 1200 hours on May 10, 11, 13, 15, and 16. On May 16, the photolysis experiments were terminated and the concentration of the test chemical and actinometer (PNAP) controls were determined in triplicate. The mean concentrations of all samples are summarized below.

Date	Conc. of Chem. (M)	Conc. of Act. (M)	Conc. of Chem. Control (M)	Conc. of Act. Control (M)
May 9	1.00×10^{-5}	1.00×10^{-5}	1.00×10^{-5}	1.00×10^{-5}
May 10	0.820×10^{-5}	0.855×10^{-5}		
May 11	0.654×10^{-5}	0.710×10^{-5}		
May 13	0.440×10^{-5}	0.515×10^{-5}		
May 15	0.299×10^{-5}	0.383×10^{-5}		
May 16	0.233×10^{-5}	0.304×10^{-5}	0.997×10^{-5}	0.996×10^{-5}

Since no significant loss of PNAP or test chemical was observed in the control samples, no adventitious processes occurred and the loss of test chemical and PNAP was only due to sunlight photolysis.

Using the above data, $\ln(C_0/C_t)$ for the test chemical and actinometer can be calculated and the results are summarized below.

<u>t (days)</u>	<u>Chemical</u>		<u>Actinometer</u>	
	<u>$C_t \times 10^5$ (M)</u>	<u>$\ln(C_0/C_t)^c$</u>	<u>$C_t \times 10^5$ (M)</u>	<u>$\ln(C_0/C_t)^a$</u>
0	1.00	0.000	1.00	0.000
1	0.820	0.199	0.855	0.157
2	0.654	0.425	0.710	0.343
4	0.440	0.821	0.515	0.664
6	0.299	1.21	0.383	0.960
7	0.233	1.46	0.304	1.19

The ratio of the rate constants, k_p^c/k_p^a , is defined by equation 12

$$\ln(C_0/C_t)^c = (k_p^c/k_p^a) \ln(C_0/C_t)^a \quad (12)$$

Using all the data (including the time point $t = 0$) and linear regression analysis, the slope is found to be 1.237 with a correlation coefficient of 0.9998. Therefore

$$(k_p^c/k_p^a) = 1.24$$

Using the molar absorptivities obtained in example 2, Section V.B.1.b., and the L_λ values for spring at 30° north latitude, Table 4, the value of $\sum \epsilon_\lambda L_\lambda$ can be calculated as follows:

$\lambda_{\text{center}}(\text{nm})$	$\epsilon_\lambda (\text{M}^{-1}\text{cm}^{-1})$	L_λ^*	$\epsilon_\lambda L_\lambda (\text{days}^{-1})$
299	1534	2.30(-4)	0.35
304	1000	2.13(-3)	2.13
309	200	7.26(-3)	1.45
314	100	1.65(-2)	1.65
319	10	2.64(-2)	0.26
323	1	2.69(-2)	0.03
340	0	3.20(-1)	0

* The units of L_λ are 2.303×10^{-3} einsteins $\text{cm}^{-2} \text{ day}^{-1}$.

$$\sum \epsilon_\lambda^c L_\lambda = 5.87 \text{ days}^{-1}.$$

For this experiment, $k_a^a(\sum \epsilon_\lambda^a L_\lambda)$ is 371 days^{-1} (Table 2). All the pertinent data are summarized below:

$$k_p^c/k_p^a = 1.24; \quad \sum \epsilon_\lambda^c L_\lambda = 5.87 \text{ days}^{-1};$$

$$\sum \epsilon_\lambda^a L_\lambda = 371 \text{ days}^{-1}; \quad \phi_E^a = 1.96 \times 10^{-4}.$$

Substituting these results into equation 10 yields

$$\phi_E^c = (1.24) \frac{(371)}{(5.87)} (1.96 \times 10^{-4})$$

$$\phi_E^c = 0.0154.$$

The rate constants for direct photolysis of test chemical in aqueous media and the half-life for water bodies and clear sky conditions for the winter and summer seasons can be calculated as follows: The values of $\sum \epsilon_{\lambda}^C L_{\lambda}$ have been calculated from example 1, Section V.B.1.b. For summer $\sum \epsilon_{\lambda}^C L_{\lambda} = 6.93 \text{ days}^{-1}$; for winter $\sum \epsilon_{\lambda}^C L_{\lambda} = 2.16 \text{ days}^{-1}$. The quantum yield for the chemical is 0.0154. Using these data in equation 4 yields

$$\text{summer: } k_{pE} = 0.0154 (6.93) = 0.107 \text{ days}^{-1}$$

$$\text{winter: } k_{pE} = 0.0154 (2.16) = 0.033 \text{ days}^{-1}.$$

These values can be substituted into equation 2 to obtain the half-lives for these two seasons.

$$\text{summer: } t_{1/2E} = \frac{0.693}{0.107} = 6.5 \text{ days}$$

$$\text{winter: } t_{1/2E} = \frac{0.693}{0.0333} = 21 \text{ days}$$

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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHT

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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PHOTOLYSIS IN AQUEOUS SOLUTION IN SUNLIGHTI. NEED FOR THE TEST

The majority of the earth's surface is covered by water in the form of oceans, seas, rivers, lakes, streams, or ponds. As a result, chemicals are likely to enter aqueous media and could then under transformation via direct aqueous photolysis.

Direct aqueous photolysis represents the transformation of a chemical substance by direct absorption of radiant energy (sunlight) into new chemicals different from their precursors. Chemical substances which are present in aqueous media photolyze at different rates depending upon the solar irradiance, the chemical substance's molar absorptivity at each wavelength of solar radiation, and its photolysis quantum yield at the wavelengths of concern. Chemical substances which photolyze rapidly under environmental conditions have relatively short lifetimes in the environment. Consequently, the chemical fate assessment may focus on the transformation products to a greater extent than on the parent compound. On the other hand, if the chemical substance is resistant to photolysis as well as to all the other possible transformation processes, the assessment should focus on the parent chemical.

A cost-effective aqueous photolysis test is needed to assess quantitatively the transformation of chemical substances in sunlight. The importance of direct photolysis in sunlight as a

transformation process for chemical substances in aqueous media in the environment can be determined quantitatively from data on photolysis rate constants and half-lives.

The aqueous photolysis test represents a screening test to allow one to determine how rapidly direct photolysis will take place in aqueous media under certain environmental conditions. If the photolysis test data indicate that photolysis is a relatively important transformation process and the initial risk assessment indicates that there is a threat to the health of humans and/or to the environment, then upper-tier tests may be required to obtain more precise aqueous photolysis data over a wide range of environmental conditions. These upper-tier tests will also be concerned with determining the identity and fate of the transformation products.

II. SCIENTIFIC ASPECTS

A. Rationale for the Selection of the Test Method

1. Historical Discussion

The scientific literature contains a number of publications dealing with the photolysis in solution of various chemical substances. Unfortunately, for one or more reasons, most of the methods and data contained in the literature are of little or no use in determining aqueous photolysis rate constants and half-lives. Reasons for this include: (1) Many of the publications

deal primarily with the mechanisms and products from direct photolysis reactions rather than photolysis rates. (2) The publications give no quantitative data on the rates of photolysis under environmental conditions, i.e., many researchers used sources of light which do not simulate sunlight or performed experiments in solvents other than air-saturated water. (3) Some studies are not based upon the fundamental laws of photochemistry. (4) Many publications report the effects of certain sensitizers which are environmentally unimportant, or the effect of other chemicals present in solution, on the chemical substance being studied. (5) Many of the studies suffer from poor experimental design, overlooking the controls necessary to make sure that only photolysis and not biodegradation, volatility, or other competing processes are taking place to remove or transform the chemical substance. The following paragraphs cite a few representative examples which illustrate the points mentioned above, as well as the publication which forms the basis for this Test Guideline.

A paper by Grunwell and Erickson (1973) deals with the photochemistry of parathion. This report is of little relevance in the evaluation of photolysis rates of chemical substances for several reasons. The main emphasis of this work is in the identification of major products formed by the photolysis of parathion rather than in the measurement of rate constants and half-lives. Photolysis was performed at three wavelengths (254, 300, and 350 nm), of which only the last two are environmentally

relevant. The chemical was dissolved in solutions of 20 percent water and either 80 percent ethanol or tetrahydrofuran (by weight), neither of which is environmentally relevant.

A paper by Langford et al. (1973) on the photolysis of nitrilotriacetic acid claims environmental relevance by working under environmental conditions. Radiation of 350 nm as well as sunlight was used. Pure water and river water were used as solvents. The authors presented no quantitative data on the rate of photolysis. Detailed quantitative measurement of the photolysis rate constant and half-life, along with adequate controls, would be necessary to make this research useful for the purposes of this Test Guideline.

Benson et al. (1971) photolyzed chlordane with both mercury arc radiation and sunlight. Acetone was used as both a solvent and photosensitizer. Sunlight photolysis was done only on the pure compound applied as a thin layer under quartz glass. The main emphasis of this work was to look at the chemical structure of the reaction products. Because rate constants were not measured, acetone was used as both a solvent and sensitizer, and a mercury arc lamp was used as radiation source, their procedures have minimal applicability as a test method to screen for environmental photolysis rates.

Su and Zabik (1972) studied the photochemistry of arylamidine derivatives in distilled and natural water. A high pressure mercury arc, filtered to remove radiation below 286nm

was used. Products of the reaction were determined but no kinetic studies were performed and no rate constants or half-lives were reported.

Mancini (1978) presented a theoretical framework for the first-order photodecomposition of picloram in aqueous solution and tested the framework using some experimental data on the photolysis of picloram in sunlight. In reality, the Mancini framework is only an empirical approach describing the rate of photolysis of picloram in sunlight.

Zepp and Cline (1977) published a paper on direct photolysis in aqueous environments with equations for the determination of direct photolysis rate constants in sunlight. This paper avoids the problems illustrated above and serves as the starting point for the development of a test method for direct photolysis in aqueous solution in sunlight. These equations translate readily obtained laboratory data into rate constants and half-lives for sunlight photolysis. Photolysis half-lives can be calculated as a function of season, latitude, time of day, depth in water bodies, and thickness of the atmospheric ozone layer. Several published papers concerning the photolysis of chemicals in sunlight have verified this method. (Wolfe et al. 1976, Smith et al. 1977, 1978, Zepp et al. 1975, 1976).

2. Selection of the Test Method

The methods in this Test Guideline were developed from a thorough review of the research literature on the experimental determination of aqueous photolysis rate constants, the principles outlined by Zepp and Cline (1977), Zepp (1978), the method developed by the U.S. Environmental Protection Agency, Office of Toxic Substances [USEPA (1979)], and by the research carried out by SRI International [Mill et al. (1981, 1982a, 1982b) and Dulin and Mill (1982)].

The proposed test method for the measurement of direct sunlight photolysis of a chemical substance in aqueous solution is based upon four fundamental criteria. These criteria are:

- (1) The test method should be based upon the fundamentals of photochemistry.
- (2) The test method should yield quantitative data on direct photolysis rates of chemical substances in aqueous media.
- (3) Sunlight should be used as the irradiation source because of its obvious environmental relevance as well as its low cost in comparison to artificial light sources (Zepp 1982).
- (4) The test method should be designed to account for transformation or chemical losses by mechanisms other than photolysis. For example, the experiments should be designed to eliminate or minimize biodegradation and volatilization and to account for hydrolysis as a factor in the estimation of test substance losses.

A simple first-tier screening test is used to estimate the direct aqueous photolysis rate constant and half-life in sunlight as a function of latitude and season of the year. An upper limit for this rate of photolysis can be obtained by assuming that photolytic transformation occurs with a quantum efficiency of unity and by equating the rate of reaction with the rate of absorption of light. The latter rate, which represents the maximum rate constant, can be estimated as a function of latitude and season of the year by combining ultraviolet-visible absorption spectral data with the appropriate solar irradiance data. The maximum rate constant can then be used to obtain the corresponding minimum half-life for photolysis in water in sunlight. If these data indicate that aqueous photolysis is an important transformation process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.) then additional tests (i.e., upper-tier tests) should be carried out to determine environmentally relevant rate constants and half-lives.

A second-tier aqueous photolysis screening test is used to determine rate constants and half-lives in the presence of sunlight. This method is divided into two phases. In phase one, the test chemical is photolyzed in sunlight in order to obtain an approximate rate constant [USEPA (1979)]. However, this method only gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis. In phase two, a standard actinometer is used to overcome this deficiency [Mill et al. (1981, 1982b) and Dulin and Mill

(1982)]. The rate constant of the standard actinometer can be adjusted so that its half-life can range from a few hours to several weeks. By using the data from phase one, the rate constant of the standard actinometer can be adjusted to match the approximate rate constant of the test chemical. Then, by simultaneously photolyzing the test chemical and the standard actinometer, the sunlight quantum yield of the test chemical can be determined. Combining this quantum yield with the test chemical spectral absorption data from the tier-one test and the appropriate solar irradiance data in water, environmentally relevant rate constants (i.e., for water bodies under clear sky conditions) can be calculated as a function of latitude and season of the year. These rate constants can then be used to determine the corresponding half-lives for photolysis of a test chemical in sunlight in aqueous media. These rate constants are in a form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

B. Development of the Test Method

1. Theoretical Aspects

The theory of the method of Zepp and Cline (1977) is briefly discussed to show that the proposed test method is based upon the fundamental criteria given in Section II.A.2. These discussions lay the foundation for the proposed method, show how the method can be used to obtain direct sunlight photolysis rate constants

and half-lives, and indicate what test conditions must be standardized in order to obtain meaningful aqueous photolysis rate data.

Zepp and Cline (1977) published a paper on the rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water body. It has been shown that in dilute solution (i.e., the absorbance of a chemical is less than 0.02 in the reaction cell at all wavelengths greater than 290 nm) at shallow depths (<0.5m), the kinetic expression for direct photolysis of a chemical at a molar concentration C is

$$-\frac{dC}{dt} = \phi_E k_a C = k_{pE} C \quad , \quad (1)$$

with $k_{pE} = \phi_E k_a \quad , \quad (2)$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, and $k_a = \sum k_{a\lambda}$, the sum of $k_{a\lambda}$ values of all wavelengths of sunlight that are absorbed by the chemical. The term k_{pE} represents the photolysis rate constant for water bodies in sunlight in the units of reciprocal time. Integrating equation 1 yields

$$\ln(C_0/C) = k_{pE} t \quad , \quad (3)$$

where C is the molar concentration of chemical at time t during photolysis and C_0 is the initial molar concentration. By measuring the concentration of chemical as a function of the time t during photolysis in sunlight, k_{pE} can be determined using equation 3. In addition, equation (3) can be solved for the condition $C_t = C_0/2$ and the half-life of the chemical is given by

$$t_{1/2} = \frac{0.693}{k_{pE}} \quad (4)$$

Furthermore, under the same conditions cited above [i.e., for homogeneous chemical solution with absorbance less than 0.02* in a reaction cell at all wavelengths greater than 290 nm and at shallow depths (less than 0.5 m)], the first-order direct photolysis rate constant, k_{pE} , is

$$k_{pE} = \phi_E \int \epsilon_\lambda L_\lambda \quad (5)$$

where ϕ_E is the quantum yield which is independent of the wavelength, ϵ_λ is the molar absorptivity in the units molar⁻¹ cm⁻¹, and L_λ is the solar irradiance in water in the units 2.303×10^{-3} einsteins cm⁻² day⁻¹ [Mill et al. (1981, 1982a, 1982b)]. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

* At an absorbance of 0.05, equation (5) is in error by only 11%. This is an acceptable error limit for a screening test and an absorbance of 0.05 has been adopted for use in this Test Guideline.

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this guideline measures rate constants in tubes (e.g., 13 x 100 mm) and the rate is faster in tubes. This is discussed in more detail in Section II.B.3.b.iii. Thus, equations 3 and 4 have to be modified to take this into account. For simplicity, the following nomenclature is used. For water bodies, the rate constant is designated as k_{pE} with the subscript E designating rates in the environment in water bodies. For tubes, the rate constant is designated as k_p . The corresponding half-lives for water bodies and tubes are $t_{1/2E}$ and $t_{1/2}$, respectively. Thus, for tubes, equation 3 and 4 can be written as

$$\ln(C_0/C) = k_p t \quad (6)$$

$$t_{1/2} = \frac{0.693}{k_p} \quad (7)$$

2. Tier 1 Test: UV-Visible Absorption Spectra -
Estimation of Aqueous Photolysis Maximum Rate
Constant and Minimum Half-life in Sunlight

A simple first-tier screening test has been developed using equation 5. As an approximation, it is assumed that the quantum yield ϕ_E is equal to one, the maximum value*. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 5 becomes

$$(k_{pE})_{\max.} = \sum \epsilon_{\lambda} L_{\lambda} \quad (8)$$

Substituting equation 8 in equation 4, the lower limit for the half-life is then given by

$$(t_{1/2E})_{\min.} = \frac{0.693}{(k_{pE})_{\max.}} = \frac{0.693}{\sum \epsilon_{\lambda} L_{\lambda}} \quad (9)$$

In order to estimate $(k_{pE})_{\max.}$, it is necessary to obtain data on the molar absorptivities of the test chemical and the solar irradiance (equation 8). These two parameters can be obtained in the following way.

*It is possible that under certain circumstances ϕ_E can be greater than 1. For example, this happens when a chain reaction occurs after the absorption of a quantum of light. However, this rarely occurs in dilute aqueous solution.

a. Determination of Molar Absorptivity

The molar absorptivity of a solute (test chemical) can be obtained from the Beer-Lambert law and the ultraviolet-visible absorption spectrum. The Beer-Lambert law states that for an absorbing solute present in water, the decrease in intensity of light with thickness $(-dI/dl)$, at a fixed wavelength λ , is proportional to the intensity I and the concentration of the solute C in the medium [Prutton and Maron (1951)]. Therefore,

$$-\frac{dI}{dl} = \epsilon'_\lambda CI \quad , \quad (10)$$

where ϵ'_λ is the proportionality constant. Equation 10 can be easily integrated to give the standard Beer-Lambert equation

$$\log_{10}(I_0/I) = \epsilon_\lambda C l \quad , \quad (11)$$

where l is the thickness of the solution or pathlength in cm, I_0 is the intensity of light at $l = 0$, I is the intensity of light at the thickness l , C is the molar concentration, and ϵ_λ is the molar absorptivity in the units molar⁻¹ cm⁻¹.

Since A_λ , the absorbance, is equal to $\log_{10}(I_0/I)$, equation 11 becomes

$$A_\lambda = \epsilon_\lambda C l \quad . \quad (12)$$

The absorbance A_λ can be obtained from the ultraviolet-visible absorption spectrum of the test chemical in aqueous solution by the methods outlined in Test Guideline CG-1050. These data will be used in equation 12 to calculate molar absorptivities for the appropriate wavelength intervals and wavelength centers where the test chemical absorbs light. These wavelength intervals and wavelength centers are listed in Table 1 of the Test Guideline (CG-6000). The method outlined in the Test Guideline CG-1050 is the standard procedure used by all laboratories for determining the molar absorptivity of a chemical in solution.

b. Solar Irradiance

The solar irradiance in water, L_λ , is related to the sunlight intensity in water and is proportional to the average light flux that is available to cause photoreaction in the wavelength interval λ over a 24-hour day. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year. The solar irradiance is discussed in more detailed in Section II.C.9. and the values are listed in Tables 3-6 in the Test Guideline (CG-6000) as a function of latitude and season of the year.

c. Estimation of the Maximum Direct

Photolysis Rate Constant and the
Corresponding Minimum Half-Life

The maximum direct photolysis rate constant is defined by equation 8. The L_λ values are chosen from Tables 3-6 in the Test Guideline (CG-6000) for the appropriate latitude and season of the year. The molar absorptivity is determined according to Section II.B.2.a. The product of ϵ_λ and L_λ is calculated at λ_{center} for each wavelength interval where ϵ_λ has a non-zero value. Then the products of $\epsilon_\lambda L_\lambda$ are summed over all wavelength intervals for $\lambda_{\text{center}} > 299\text{nm}$. This sum is $\sum \epsilon_\lambda L_\lambda$ and is equal to $(k_{\text{pE}})_{\text{max}}$. $(k_{\text{pE}})_{\text{max}}$ is substituted into equation 9 to obtain the minimum half-life.

3. Tier 2 Test: Aqueous Photolysis in Sunlight

a. Phase 1 Test

The phase one aqueous photolysis test is based on the method developed by the U.S. Environmental Protection Agency, Office of Toxic Substance [USEPA (1979)] using equations 6 and 7. By measuring the concentration of chemical as a function of time t during photolysis in sunlight, k_p^C can be determined. This rate constant is valid for the latitude and season of the year in which the photolysis experiments were carried out. The half-life can then be calculated using k_p^C in equation 7. This method only

gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis. This data will be used in the phase two aqueous photolysis screening test.

b. Phase 2 Test

The phase two aqueous photolysis sunlight photolysis test method is based on the research carried out at SRI International [Mill et al. (1981, 1982b) and Dulin and Mill (1982)].

i. Introduction

The phase one test method has a limitation since it does not measure sunlight intensities incident on the sample during photolysis. SRI carried out a study on the use of insolation methods for measuring solar intensity (e.g, radiometry, photometry, and actinometry). The most suitable method for measuring sunlight intensity was found to be actinometry. Radiometry is used to measure total solar flux, but because radiometry involves energy rather than photon flux, it is of limited value for estimating photoselective photochemical reactions. Photometers, based on photocells and photomultipliers, have several advantages over radiometers, the most important being that they are sensitive to photon flux rather than energy flux. However, sensitive photometers, using digital pulse-counting methods, are relatively expensive and fragile. Chemical actinometry offers advantages over

instrumental methods. Actinometry conforms to the geometry of the reaction vessel and measures the actinic flux under known sensitivity conditions. Thus, in general, actinometry, to measure solar intensity, is simple to use in aqueous photolysis experiments. The main complication in using actinometry in outdoor experiments is that the sun is not a monochromatic source, and the absorption of light by the actinometer and chemical rarely match. Therefore, a series of actinometers would be needed to match the light absorption of the test chemical in the wavelength region $\lambda > 290\text{nm}$. Fortunately, the most important wavelength region for sunlight photolysis is at 290-400nm, where the high energy sunlight is concentrated, and a large number of chemicals absorb light in this region. Therefore, the most useful actinometer is one which absorbs light in the region 290-400nm.

ii. Development of a Sunlight Actinometer

Listed below are several criteria for chemical and spectral properties which should be given high priority in selecting sunlight actinometers. The ideal actinometer should:

- (1) Absorb light moderately and uniformly in the solar region but be stable in room light;
- (2) have a quantum yield, ϕ , which is wavelength independent;
- (3) have a quantum yield that is independent of dissolved oxygen and concentration of the actinometer;

- (4) have an adjustable half-life ranging from several hours to 90 days;
- (5) have photolysis products that do not interfere with photolysis, either chemically or spectrally;
- (6) have simple, accurate, analytical methods available for its analysis;
- (7) be very stable in water in the dark;
- (8) be moderately soluble in water and relatively nonvolatile; and
- (9) have little or no temperature dependence of ϕ .

Based on a review of the photochemical literature, several hundred examples of photochemical reactions were found, many of which might fulfill some of the criteria established for the ideal actinometer. To simplify the task, the review was restricted to photoreactions that are bimolecular and are therefore amenable to changes in the rate (i.e., to changes in the value of ϕ) with changes in concentration of one photoinert reactant. Nucleophilic substitutions constituted one such class.

The most successful actinometer developed to date with a variable quantum yield is the system composed of p-nitroacetophenone (PNAP) and pyridine (PYR) [Mill et al. (1981, 1982b) and Dulin and Mill (1982)]. Laboratory experiments at 313nm and 25°C indicated that the quantum yield for transformation of PNAP depended linearly on the molar concentration of pyridine, at a fixed concentration of PNAP. Regresson analysis of the quantum

yield as a function of the molar concentration of pyridine [PYR] up to a concentration of 0.1M (at a fixed concentration of PNAP equal to $1.00 \times 10^{-5}M$) gave the relationship

$$\phi_E^a = 0.0169[\text{PYR}] \quad , \quad (13)$$

with a correlation coefficient greater than 0.99. It should be noted that in each experiment, PNAP exhibited good first-order kinetics to 75% transformation. At pyridine concentrations greater than 0.1M, nonlinearity was observed. Hence, equation 13 is only valid to a 0.1M concentration of pyridine. Further laboratory experiments were carried out at 366nm and 25°C. The data indicated that ϕ_E^a was essentially the same, within experimental error, at 313 nm and 366nm.

To determine the temperature dependence of the PNAP/PYR actinometer, photolysis rates were measured at 43°C and then compared to the rates at 25°C. The rate of photolysis slowed down by 12% at 43°C. Using the standard Arrhenius equation, the energy of activation was found to be -1.3kcalories. Therefore, in the range 1°C to 45°C, the photolysis rate constant of the PNAP/PYR actinometer will be within $\pm 25\%$ of its value at 25°C. Thus, the rate constant for this actinometer, and hence its quantum yield, is not very sensitive to changes in temperature.

The ultraviolet-visible absorption spectrum of PNAP was obtained in an aqueous solution containing 1% acetonitrile [Mill et al. (1982b)]. The molar absorptivities were calculated using

the Beer-Lambert law as a function of wavelength (i.e. at the wavelength centers listed in Table 1 of the Test Guideline) and the results are summarized in Table 1. PNAP shows absorption of light in the solar region $\lambda > 290\text{nm}$ to 430nm with strong absorption in the region around 300nm . As discussed in Section II.B.3.b.i., this actinometer is extremely useful since a large number of chemicals absorb light in this region.

Table 1. MOLAR ABSORPTIVITIES OF PNAP

$\lambda_{\text{center}} \text{ (nm)}$	$\epsilon_{\lambda}^a \text{ (M}^{-1}\text{cm}^{-1}\text{)}$
299	3300.0
304	2700.0
309	2346.0
314	1947.0
319	1577.0
323	1303.0
340	525.0
370	130.0
400	19.8
430	1.1

An important variable for the PNAP/PYR actinometer is the absorption rate constant k_a^a , in days^{-1} , and is defined by the equation

$$k_a^a = \int \epsilon_{\lambda}^a L_{\lambda} \quad , \quad (14)$$

where ϵ_{λ}^a is the molar absorptivity of PNAP and L_{λ} is the solar irradiance in water. The molar absorptivities of PNAP are listed in Table 1. Using this data with the L_{λ} values listed in Tables

3-6 of the Test Guideline (CG-6000), k_a^a has been calculated as a function of latitude and season of the year in the northern hemisphere. The results are summarized in Table 2 of the Test Guideline. This parameter is essential to carrying out the phase two aqueous photolysis experiments.

iii. Geometry of the Reaction Vessel

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this Test Guideline measures rate constants (k_p) in tubes (e.g., 13 x 100mm). Such a reaction vessel receives radiation from all directions, due to scattering of the light off the walls, in contrast to a water body (e.g., a lake) which receives sunlight only from above. It is expected, therefore, that photoreactions would be faster in tubes. This was verified experimentally [Mill et al. (1982b)]. Laboratory experiments were carried out in tubes and dishes using the actinometers p-nitroacetophenone/pyridine, p-nitroanisole/pyridine, and rose bengal. The experiments using dishes represented a water body. All the data indicated that the ratio of rate constants of tubes to dishes was 2.2 ± 0.3 . Thus,

$$k_p = \gamma k_{pE} \quad , \quad (15)$$

with $\gamma = 2.2 \pm 0.3$. The factor γ is called the geometry factor. For tubes, therefore, $\gamma = 2.2$ while for water bodies $\gamma = 1.0$.

iv. Phase 2 Test Method

In phase two, a standard PNAP/PYR actinometer is used to measure sunlight intensities incident on the sample during photolysis. The rate constant for this actinometer, k_p^a , can be adjusted to match the approximate rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the quantum yield of the actinometer, the rate constant can be adjusted according to equation 13.

Using equations 5 and 15, the rate constant in tubes is given by

$$k_p = \gamma \phi_E \sum \epsilon_{\lambda} L_{\lambda} \quad (16)$$

Therefore, the rate constants for the actinometer, k_p^a , and the test chemical, k_p^c , in tubes are

$$k_p^c = \gamma \phi_E^c \sum \epsilon_{\lambda}^c L_{\lambda} \quad (17)$$

$$k_p^a = \gamma \phi_E^a \sum \epsilon_{\lambda}^a L_{\lambda} \quad (18)$$

Since the phase two test method involves the simultaneous photolysis of test chemical and actinometer in the same size tubes, equation 18 can be divided into equation 17. Carrying out this operation and solving for ϕ_E^c yields

$$\phi_E^c = \frac{k_p^c}{k_p^a} \cdot \frac{\sum \epsilon_{\lambda}^a L_{\lambda}}{\sum \epsilon_{\lambda}^c L_{\lambda}} \phi_E^a \quad (19)$$

The quantum yield of the test chemical, ϕ_E^c , can be determined in the following way.

(1) Determination of the ratio (k_p^c/k_p^a) .

Using equation 6, the equations for the test chemical and actinometer in tubes are

$$\ln(C_o/C_t)^c = k_p^c t \quad (20)$$

$$\ln(C_o/C_t)^a = k_p^a t \quad (21)$$

Since the phase two test method involves the simultaneous photolysis of the test chemical and actinometer in the same size tubes, equation 20 can be divided by equation 21. Carrying out this operation and rearranging yields

$$\ln(C_o/C_t)^c = (k_p^c/k_p^a) \ln(C_o/C_t)^a \quad (22)$$

$\ln(C_o/C_t)^c$ is a linear function of $\ln(C_o/C_t)^a$ with a slope given by

$$\text{slope} = (k_p^c/k_p^a) \quad (23)$$

The most precise and easiest method of determining the slope is by linear regression analysis on the experimental data.

Therefore, by measuring the concentration of test chemical and actinometer at the same time as a function of time, these data can be fitted to equation 22 by linear regression analysis to give the best straight line. The slope, the ratio of the rate constants, is given by equation 23.

- (2) The term $[\epsilon_{\lambda}^a L_{\lambda}]$ for the actinometer has been tabulated as a function of latitude and season of the year [Table 2 in the Test Guideline].
- (3) The term $[\epsilon_{\lambda}^c L_{\lambda}]$ for the test Guideline can be obtained from the experimentally measured molar absorptivities (Section II.B.2) and the values of L_{λ} [listed in Tables 3 to 6 in the Test Guideline as a function of latitude and season of the year].
- (4) ϕ_E^a can be determined from equation 13 at the molar concentration of pyridine used in the standard actinometer.

With the value of ϕ_E^c , ϵ_{λ}^c , and the appropriate L_{λ} values, k_{pE} for the test chemical can be calculated as a function of latitude and season of the year using equation 5. The corresponding half-life can be calculated using k_{pE} in equation 4. These rate constants and half-lives correspond to sunlight photolysis in water bodies under clear sky conditions and at shallow depths.

Photolysis experiments were carried out in the laboratory using a merry-go-round reactor (MGRR) at 313 nm and in sunlight using the test chemicals dibenzothiophene, nitrobenzene, and p-methoxyacetophenone [Mill et al. (1982b)]. The data was used to calculate the laboratory quantum yield at 313nm, $\phi(313\text{nm})$, the sunlight quantum yield, ϕ_E^C , the half-life calculated from the laboratory data, $t_{1/2}(\text{calc.})$, and the half-life in sunlight, $t_{1/2}(\text{obs.})$. These data are summarized in Table 2. Comparison of the corresponding quantum yield data and half-life data for the laboratory experiments with those in sunlight indicated that the results are in good agreement confirming the phase two test method.

Table 2. CALCULATED AND OBSERVED QUANTUM YIELDS AND ENVIRONMENTAL PHOTOLYSIS HALF-LIVES FOR SOME TEST CHEMICALS

Chemical	$\phi(313\text{nm})$	ϕ_E^C	$t_{1/2}(\text{calc.})$ days	$t_{1/2}(\text{obs.})$ days
Dibenzothiophene	5.0×10^{-4}	7.3×10^{-4}	9.1	6.4
Nitrobenzene	1.8×10^{-4}	1.9×10^{-4}	19.8	19.3
P-Methoxyaceto- phenone	9.5×10^{-4}	1.3×10^{-4}	11.9	12.0

4. Summary

This screening test has been designed to determine the molar absorptivity of a test chemical, ϵ_λ , and its quantum yield, ϕ_E^C . These parameters can be used to determine environmental rate constants at low absorbance and shallow depths in pure water

as a function of latitude and season of the year. Tables of solar irradiance (Tables 3-6) have been included in this Test Guideline to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp and Cline (1977) have written a computer program to calculate the rates of photolysis as a function of depth in water, in natural waters as a function of the attenuation coefficient of the water (λ), the average ozone layer thickness that pertains to the season and location of interest, and as a function at latitude and season of the year. The computer program is available on request. [R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.]

This Test guideline represents a screening test to obtain the quantum yield of a chemical. Since it does not require sophisticated (and expensive) photochemical equipment, the quantum yield can be obtained easily and at a modest cost. However, the quantum yield can be obtained more precisely in the laboratory using special photochemical equipment and monochromatic light by procedures described by Zepp (1978) and Mill et al. (1982a). A quantum yield obtained by one of these procedures is acceptable as a replacement for the determination of the quantum yield in sunlight. In the near future, an upper-tier test will be published as a Test Guideline in NTIS which will give a detailed procedure for determining the quantum yield in the laboratory using a monochromatic light source.

C. Applicability and Specificity

This test method is applicable to all chemicals which have uv-visible absorptions in the range 290-800nm. Solar radiation reaching the earth's surface has a sharp cutoff at a wavelength of approximately 290nm due to the absorption by ozone [Leighton (1961), Peterson (1976), Zepp and Cline (1977), Demerjian (1980)]. the long wavelength limit is set by thermochemistry since light of wavelength greater than 800nm is not of sufficient energy to break chemical bonds of ground state molecules [Calvert and Pitts (1966), Benson (1976)]. Photolysis does not occur unless there is absorption of radiant energy. This is the direct consequence of the Grotthus-Draper law, the first law of photochemistry. If a chemical in aqueous solution only absorbs light at wavelengths below 290nm it will not undergo direct photolysis in sunlight. A few examples of chemicals that only absorb light below 290nm and therefore should not be tested by this Test Guideline are alkanes, alkenes, alkynes, saturated alcohols, and saturated acids. It is possible that some chemicals will absorb radiation mainly at wavelength below 290nm but may have an absorption tail that extends above 290nm. Photolysis experiments should be carried out for these chemicals.

This Test Guideline is only applicable to pure chemicals and not to the technical grade. Overestimates of molar absorptivities usually occur when technical substances are tested because the impurities frequently absorb in the same spectral region as the pure chemical.

D. Rationale for the Selection of the Test Conditions

1. Special Laboratory Equipment

All the laboratory equipment needed to determine the uv-visible absorption spectrum of a test chemical in aqueous solution is listed in the references in Test Guideline CG-1050. The equipment required to carry out this test is standard equipment which is commonly used in most spectroscopic laboratories.

In order to carry out the photolysis experiments, special tubes are necessary to contain the reaction solutions during photolysis. Reaction tubes with 11mm inside diameter are recommended as they are inexpensive, easy to seal to prevent volatilization of the chemical, and can be easily mounted on a rack in sunlight. Disposable culture tubes (13 x 100mm) with Teflon-lined screw caps are readily available from commercial laboratory supply companies. For some chemicals, it may be difficult to determine their concentrations in the small volumes present in 11mm i.d. tubes. For such chemicals, the use of larger reaction vessels is permissible as long as the pathlength is less than 0.5 meters. This is necessary in order that first-order kinetics are obeyed (Section II.B.1). Furthermore, the cell walls should be relatively thin to minimize the loss of sunlight through the cell walls. Reaction tubes of either quartz or thin-walled borosilicate glass may be used. If a chemical absorbs strongly at $\lambda < 340\text{nm}$, then quartz tubes should be

used. Quartz will transmit 100% of the sunlight in the region 290-340nm while borosilicate glass will absorb some of this light. The absorption spectrum of the chemical in aqueous solution, as measured by Test Guideline CG-1050, can be used to determine which type of glass to use.

It is extremely important that all reaction tubes be capable of being sealed (without the use of grease) and be filled as completely as possible to prevent volatilization of test chemical or water (see Section II.C.6). Screw caps lined with Teflon inserts are recommended. Grease should be avoided since hydrophobic chemicals might adsorb to it.

2. Purity of Water

Pure water is used because dissolved impurities could sensitize or otherwise affect the rate of photolysis. In addition, the water needs to be sterile because bacteria may consume or alter the chemical substance during the prolonged periods of testing which may occur in the course of a rate determination. Thus, pure water [e.g., ASTM Type II A (ASTM 1979)], or an equivalent grade, is recommended in this Test Guideline. Furthermore, it is important that the water be saturated with air prior to preparation of the test and control solutions to simulate environmental conditions. Air-saturated water can be easily prepared by allowing the water to equilibrate in a vessel plugged with sterile cotton.

3. Sterilization

Sterilization is necessary to kill the bacteria and therefore eliminate or minimize biodegradation which could interfere with the photolysis rate determination. The presence of bacteria in either the test solutions or controls may cause biodegradation of the test substance, especially when the photolysis experiments are carried out over a long period of time. This will introduce an error in the concentration of the test chemical. Thus, it is extremely important to use aseptic conditions in carrying out all photolysis experiments to minimize biodegradation. Glassware can be sterilized easily in an autoclave or by use of any other suitable non-chemical method.

4. Concentration of Solution

It is extremely important that solutions of chemical substances used in this Test Guideline be prepared at low concentrations in order to approximate environmental conditions and to allow first-order kinetics assumptions to apply (see Section II.B.1.). Furthermore, an absorbance of 0.05 has been adopted in this Test Guideline since solutions with this absorbance can be easily achieved in the laboratory. This absorbance limit yields an 11% error, an acceptable error for a screening test (Section II.B.1.).

If the chemical substance is too difficult to dissolve in pure form to permit reasonable handling and analytical procedures, then a test solution may be prepared by first

dissolving a chemical in reagent grade acetonitrile. The final acetonitrile concentration in the test solution should be no more than one volume percent in order to avoid solvent effects (Smith et al. 1977, 1978). Acetonitrile was chosen as a solvent as it is soluble in water, is non-polar and thus effective in dissolving many substances which are insoluble in water, it does not absorb radiation over the wavelength range of 290 to 800nm, and it causes minimal solvent effects (i.e., minimum shifts in bands and changes in absorbance) for test substances.

5. pH Effects

The molecular structure of a chemical substance which ionizes or protonates is a function of the pH. As a result, the absorption spectrum and consequently the rate of photolysis may change with pH. The recommended procedure is to determine the uv-visible spectra under conditions in which only one species strongly predominates in solution. This can be accomplished by preparing buffered solutions containing the test chemical at pHs at least 2 units above the pK_a and at least 2 units below the pK_a . For example, the pK_a for phenol is 9.89. At a pH below 7.89, the protonated form of the phenol strongly predominates; at a pH of greater than 11.89, the phenoxide form of the phenol strongly predominates. The reaction quantum yield for ionizable compounds should also be determined in buffered solutions, where only one species strongly predominates. Thus, photolysis experiments should be carried out at the same pHs used to obtain the spectrum of each species.

Since buffers could influence the rate of photolysis, the recommended buffers for use in this Test Guideline were carefully chosen to be transparent to radiation between 290 and 800nm and should be kept at very low concentrations to avoid buffer effects which may cause transformation of the substance by, for example, catalysis.

6. Control Solution

Undetected loss of a test substance through volatilization, hydrolysis, or other processes during the course of the photolysis experiment will result in the determination of erroneously large rate constants for aqueous photolysis. Therefore, for volatile chemical substances, it is important that the reaction vessels and control vessels be filled as completely as possible and sealed in order to avoid evaporative losses. To correct for possible losses, control solutions of test substance, in darkened vessels, are placed side by side with the photolysis vessels and the contents of the control vessels are analyzed at the end of the experiment. In this way, the loss of test chemical for processes other than photolysis may be determined and eliminated or accounted for in determining k_p . For simplicity, if the loss of chemical in the control is small, one can calculate a first-order loss, k_{loss} , and subtract it from $(k_p)_{obs}$ to give the corrected direct photolysis rate constant k_p . If hydrolysis is found to be significant, hydrolysis studies should be carried out first to define precisely the kinetics of this process [Test Guideline CG-5000].

7. Absorption Spectrum as a Criterion for
Performing the Aqueous Photolysis Test

The test method is applicable to all chemicals that have uv-visible absorptions in the range 290-800nm (section II.C). Thus, the uv-visible absorption spectrum of a chemical in aqueous solution will give a good indication of whether it would be useful to carry out sunlight aqueous photolysis tests.

8. Sunlight Actinometer

In order to quantify the rate of photolysis more precisely, it is necessary to measure the sunlight intensity incident on the sample during photolysis. A standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) has been developed and the details have been discussed in Section II.B.3.b.ii. According to equation 5, the rate constant is a function of the quantum yield. Furthermore, the quantum yield can be adjusted by varying the molar concentration of pyridine according to equation 13. Hence, by varying the pyridine concentration, the PNAP photolysis rate constant can be adjusted so that the half-life can range from several hours to several weeks. In order to make the actinometer operable, it is necessary that $[PYR] > 10 [PNAP]$ and $< 0.1M$. Therefore, the concentration of PNAP must be approximately $1 \times 10^{-5}M$. In the recommended actinometer formulation, the concentration of PNAP is set at $1.00 \times 10^{-5}M$. The concentration required to adjust the rate of the actinometer

to equal the rate of the test chemical can be derived as follows. Since it is necessary to adjust the rate of photolysis of the chemical to that of the test chemical, then

$$k_p^c = k_p^a \quad . \quad (23)$$

According to equation 15, the rate constant for the actinometer in tubes is

$$k_p^a = 2.2 k_{pE}^a \quad . \quad (24)$$

However, k_{pE}^a for the actinometer can be obtained from equation 5,

$$k_{pE}^a = \phi_E^a \epsilon_\lambda^a L_\lambda \quad , \quad (5)$$

and since k_a^a for the actinometer is $\epsilon_\lambda^a L_\lambda$,

$$k_{pE}^a = \phi_E^a k_a^a \quad . \quad (25)$$

Substitution of equation 25 into equation 24 yields

$$k_p^a = 2.2 \phi_E^a k_a^a \quad . \quad (26)$$

According to equation 13, $\phi_E^a = 0.0169[\text{PYR}]$. Substituting this result in equation 26 yields

$$k_p^a = 0.0372[\text{PYR}] k_a^a \quad . \quad (27)$$

Finally, substituting equation 27 into equation 23 and solving for [PYR] yields

$$[\text{PYR}] = 26.9 \left(\frac{k_p^c}{k_a} \right) \quad (28)$$

The term k_p^c is the sunlight rate constant for the test chemical in tubes and the variable $k_a (= \epsilon_\lambda^a L_\lambda)$ for the actinometer can be obtained from Table 2 of the Test Guideline.

For 1 liter of solution, the mass of pyridine required to adjust the rate of the actinometer is

$$\text{mass(grams)} = \text{F.W.} [\text{PYR}] \quad (29)$$

where F.W. = 79.10 g/mole, the formula weight of pyridine. The density of pyridine is 0.9819 g/mL at 20°C [Weast (1973-1974)]. Thus, the volume of pyridine at 20°C required to equal this mass is

$$V(\text{mL}) = 80.6 [\text{PYR}] \quad (30)$$

8. Solar Irradiance Data

In order to calculate the quantum yield of the test chemical, ϕ_E^c , and then calculate k_{pE}^c and $t_{1/2}^c$, it is necessary to use solar irradiance data L_λ . The theoretical basis for the L_λ data is discussed in the following paragraphs.

In a water body exposed to the sun, the intensity of light passing into a water body is composed of light directly from the sun ($I_{d\lambda}$) and the sunlight from the sky ($I_{s\lambda}$). Leighton (1961) discussed the calculation and contribution of direct and sky light to the total intensity of sunlight filtering down through the troposphere at a point on the earth's surface as a function of the zenith angle Z , Figure 1. Zepp and Cline (1977) extended the treatment to the behavior of $I_{d\lambda}$ and $I_{s\lambda}$ that enter a water body, Figure 1. Part of the theoretical treatment has been summarized in Section II.B.1 and the following discussion extends this treatment. According to equation 2

$$k_{pE} = \phi_E k_a = \phi_E [k_{a\lambda}] \quad (2)$$

Futhermore, Zepp and Cline showed that

$$k_{a\lambda} = 2.303 \epsilon_\lambda j^{-1} Z_\lambda \quad (31)$$

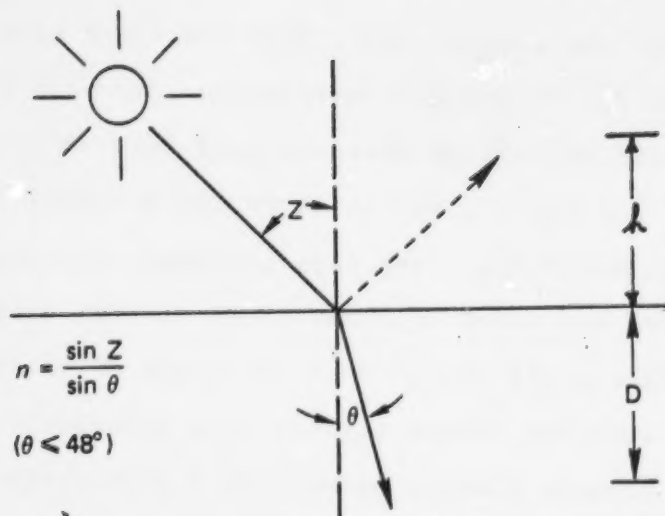
$$\text{where} \quad Z_\lambda = I_{d\lambda} \sec \theta + 1.2 I_{s\lambda} \quad (32)$$

In equation 31, the term ϵ_λ is the molar absorptivity of a chemical in aqueous solution, j is a constant which converts the intensity units into units that are compatible with the concentration units of C for the chemical (i.e., $j = 6.02 \times 10^{20}$ when C is the molar concentration of the chemical). In equation 32, $I_{d\lambda}$ and $I_{s\lambda}$ are the direct and sky sunlight intensity in photons $\text{cm}^{-2} \text{sec}^{-1}$, and θ is defined by the refractive index of

water, Figure 1. Z_λ is the actinic irradiance in water at shallow depths in the units of photons $\text{cm}^{-2} \text{sec}^{-1}$. Zepp and Cline (1977) tabulated Z_λ values for 40°N latitude as a function of midseason dates for spring, summer, winter, and fall. Substitution of equation 31 into equation 2 yields

$$k_{pE} = 2.303j^{-1} \phi_E \sum \epsilon_\lambda Z_\lambda \quad (33)$$

Based on their theoretical work, Zepp and Cline have written a computer program for 39 specific wavelength intervals from 296.3 to 825nm which calculates instantaneous rate constants at specific times of day for a given latitude and midseason date and summed them up to obtain k_{pE} . The term midseason date used by Zepp and Cline does not refer to a day midway in the season, but rather to a day during the season when the angle of declination of the sun is at specific values (listed in a subsequent paragraph). The program further calculates a photolysis rate constant k_{pE} , which is averaged over a 24-hour calendar day in the units of days^{-1} . Required data inputs are: (1) molar absorptivities of the chemical at wavelengths greater than 297.5nm; (2) the refractive index of the medium (e.g., for water $n = 1.34$); (3) the attenuation coefficient, α_λ , of the medium (e.g., α_λ for distilled water or for natural water bodies); (4) the quantum yield, ϕ_E^C , of the chemical; (5) the solar declination, solar right ascension, and sidereal time for the date of interest obtained from the American Ephemeris and



Z = Angle of incidence (solar zenith angle in case of direct sunlight)
 θ = Angle of refraction —
 n = Refractive index of water

FIGURE 1 REFLECTION AND REFRACTION OF A LIGHT BEAM PASSING FROM ATMOSPHERE INTO A WATER BODY
 (From Zepp and Cline, 1977)

Nautical Almanac [AENA (1982)]; (6) the latitude and longitude; and (7) the average ozone layer thickness that pertains to the season and location of interest.

For routine computations, the program calculates rate constants k_{pE} and half-lives for midseason dates corresponding to solar declinations of $+10^\circ$ for spring, $+20^\circ$ for summer, -10° for fall, and -20° for winter, as a function of local time of day, longitude, and latitude for 0° to 90°N in 10° increments.

To obtain the L_λ data, Mill et al. (1982a), used the Zepp and Cline computer program with $\epsilon_\lambda = 1$, $\phi = 1$, and the absorption coefficients α_λ for pure water. The L_λ values were obtained for the midseasons of winter, spring, summer, and fall for the latitudes 20° to 50°N and these data are listed in Tables 3 to 6 of the Test Guideline. The L_λ values correspond to the 1982 midseason dates of January 21, April 16, July 24, and October 20 [AENA (1982)]. The values of L_λ are in the units of 2.303×10^{-3} einsteins $\text{cm}^{-2} \text{day}^{-1}$. The L_λ values are applicable to clear sky conditions, water bodies, shallow depths, and for chemicals with very weak absorbance in pure water.

The solar irradiance Z_λ data pertain to midday, midseason, values in the units of photons $\text{cm}^{-2} \text{sec}^{-1}$ while the solar irradiance L_λ data represent day averaged, midseason, values in the units of 2.303×10^{-3} einsteins $\text{cm}^{-2} \text{day}^{-1}$. The L_λ data are used in equation 5 to calculate day-averaged, midseason, direct photolysis rate constants. The Z_λ data are used in equation 33 to calculate midday, midseason, direct photolysis rate constants.

10. Geometry of the Reaction Vessel

The method of Zepp and Cline (1977) and the method of Mill et al. (1982a) are applicable to sunlight incident on a water surface such as a natural water body. However, the method developed in this Test Guideline measures rate constants in tubes (e.g., 13 x 100mm). Such a reaction vessel receives radiation from all directions, due to scattering of the light off the walls, in contrast to a water body such as a lake which receives sunlight only from above. It is expected, therefore, that photoreactions would be faster in tubes. This was verified experimentally [Mill et al. 1982b)] and this has been discussed in detail in Sections II.B.3.b.iii. For these experiments, tubes are the simplest and easiest reaction vessels to use since they can be sealed easily to minimize or eliminate volatilization and can be handled easily. Hence, this Test Guideline recommends the use of tubes as reaction vessels and the method has been modified to take into account the increased rate in tubes (equation 15).

11. Chemical Analysis of Solutions

The analytical techniques employed in the determination of the concentration of the test substances are left to selection by the tester. This is in recognition of the many different techniques available and the practical advantage of being able to make particular use of one of the properties of the substance; e.g., the NMR or UV spectrum of the substance, or its chromatographic behavior. Analytical techniques that permit the

determination of the test compound to the exclusion of impurities or photolysis reaction products are recommended to the extent practicable. Therefore, chromatographic techniques are particularly desirable. Whenever practicable, an analytical procedure should be used which has a precision of $\pm 5\%$ or better. The specific technique which is utilized should be adequately described.

The p-nitroacetophenone in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a C_{18} reverse-phase column and a uv detector set at 280nm. The mobile phase in volume percent is 2.5% acetic acid, 50% acetonitrile, and 47.5% water which is passed through the column at a flow rate of 2 mL/minute. This analytical procedure was specifically developed to analyze for PNAP, especially at high pyridine concentrations (i.e., for pyridine concentrations close to 0.1M) [Mill et al. (1982b)].

12. Outdoor Experimental Conditions

It is important that the photolysis reaction vessels containing the chemical substance be placed in an area free from shade and reflections and on a black, non-reflecting, background to insure that they only receive direct and sky radiation from the sun. The reaction vessels should be tilted at 30° from horizontal with the upper end pointing due north in the northern hemisphere so that they present a large surface area and minimum pathlength to the sun and create minimal internal reflections.

It is recommended that the photolysis experiments be carried out during a frost-free time of the year (i.e., May, June, July, August, or September in the northern hemisphere--temperature permitting). This period of time was chosen because the solar intensity is a maximum and consequently the rate of photolysis will be a maximum. Thus, the kinetics of photolysis will be easier to follow, especially for chemicals that photolyze slowly. Furthermore, in many parts of the United States, the temperature falls below 0°C during the winter months. Therefore, if the photolysis experiments are carried out in the winter, the dilute aqueous reaction solution would freeze, the tubes would break, and the samples would be destroyed. It may be possible to avoid this problem by placing the reaction tubes in a housing transparent to the appropriate wavelengths of radiation and thermostated to control the temperature at $25 \pm 5^\circ\text{C}$. Thus, with this device it may be possible to carry out photolysis experiments at any time of the year. However, transmission of sunlight through the housing could present a difficult problem to solve.

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IV. APPENDIX: GLOSSARY OF IMPORANT SYMBOLS

PYR	Pyridine
PNAP	p-Nitroacetophenone
λ	Wavelength λ
A_{λ}	Absorbance at wavelength λ .
a	Actinometer (composed of PNAP/PYR).
ϵ_{λ}^C	Molar absorptivity of a chemical C.
ϵ_{λ}^a	Molar absorptivity of the actinometer.
l	light pathlength; the distance traveled by a beam of light passing through the system.
ϕ_E^C	Sunlight reaction quantum yield of chemical C in water.
ϕ_E^a	Sunlight reaction quantum yield of the actinometer in water.
[C]	Molar concentration of chemical C.
[PYR]	Molar concentration of pyridine.
$-d[C]/dt$	Direct photolysis rate of chemical C.
k_{pE}	Direct photolysis sunlight rate constant in water bodies in the environment.
$(k_{pE})_{max.}$	Maximum direct photolysis sunlight rate constant in water bodies in the environment.

- k_p^c Direct photolysis sunlight rate constant of chemical C in water in tubes.
- k_p^a Direct photolysis sunlight rate constant of the actinometer in water in tubes.
- $k_{a\lambda}$ Specific light absorption of a photoreactive chemical at a low concentration and at wavelength λ .
- k_a Specific light absorption rate constant integrated over all wavelengths absorbed by the chemical.
- k_a^a Specific light absorption rate constant integrated over all wavelengths absorbed by the actinometer.
- $t_{1/2}$ Sunlight half-life of a chemical in water in tubes.
- $(t_{1/2})_{\min}$ The minimum sunlight half-life of a chemical in water bodies in the environment.
- I The numbers of photons of light of wavelength λ in the system per cm^2 per second.
- L_λ Solar irradiance in water in the units einsteins cm^{-2} day $^{-1}$.
- γ The geometry factor which represents the ratio of the rate constants in tubes (k_p) to the rate constant in water bodies in the environment (k_{pE}).

CG-7000
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GAS PHASE ABSORPTION CROSS SECTION AND SUNLIGHT PHOTOLYSIS

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

DISCLAIMER

Certain commercial equipment, instruments, and materials are identified in this Test Guideline in order to specify adequately the experimental procedure. In no case does the identification of a manufacturer imply endorsement by the U.S. Environmental Protection Agency.

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GAS PHASE ABSORPTION CROSS SECTION AND SUNLIGHT PHOTOLYSISI. INTRODUCTIONA. Background and Purpose

Numerous chemicals enter the atmosphere from a variety of sources. For example, chemicals enter the atmosphere as a result of the burning of coal, from the combustion of gasoline in cars and diesel fuel in trucks, and from the release of volatile organic chemicals during manufacture, processing, use, and disposal. Pesticides, applied from airplanes, enter the atmosphere directly and volatilize from soils and water bodies. Chemical pollutants present in the atmosphere can undergo photochemical transformation in the environment by direct photolysis in sunlight. Quantitative data in the form of rate constants and half-lives are needed to determine the importance of direct photolysis of pollutants in the atmosphere. This Test Guideline describes a first-tier screening level test method to estimate the maximum direct photolysis rate constant and minimum half-life of chemicals in the atmosphere in sunlight as a function of latitude and season of the year in the northern hemisphere.

B. Definitions and Units

- (1) "Radiant energy", or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.
- (2) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_0) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ . Thus, $A_\lambda = \log_{10} (I_0/I)$.
- (3) The "Beer-Lambert law" states that the absorbance of a chemical in the gas phase, at a fixed wavelength, is proportional to the thickness of the absorbing material (ℓ), or the light pathlength, and the concentration of the absorbing species (C).
- (4) "Cross section (σ'_λ)" is defined as the proportionality constant in the Beer-Lambert law. Thus $A_\lambda = \sigma'_\lambda C \ell$, where A_λ is the absorbance, C is the concentration in molecules per cm^3 and ℓ is the pathlength in cm. The units of the cross section σ'_λ are $\text{cm}^2 \text{ molecule}^{-1}$. Numerical values of the cross section depend upon the nature of the absorbing species.
- (5) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.

- (6) The "half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical being photolyzed to be reduced to one-half its initial value.
- (7) The "sunlight direct photolysis rate constant (k_{PE})" is the first-order rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical in the gas phase in sunlight.
- (8) The "actinic solar irradiance in the atmosphere ($J_{\Delta\lambda}$)" is related to the sunlight intensity in the atmosphere and is proportional to the average light flux (in the units of $\text{photons cm}^{-2} \text{ day}^{-1}$) that is available to cause photoreaction in the wavelength interval $\Delta\lambda$ over a 24-hour day at a specific latitude and season date. It is the irradiance which would be measured by a weakly absorbing spherical actinometer exposed to sun and sky radiation from all directions.
- (9) "The Grotthus-Draper law", the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.
- (10) The "Stark-Einstein law", the second law of photochemistry, states that only one molecule is activated to an excited state per photon or quantum of light absorbed.

- (11) The "reaction quantum yield (ϕ_λ)" for an excited state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ . It is the ratio of a number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ .
- (12) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

C. Principle of the Test Method

For weak absorbance of a chemical in the atmosphere, the first-order direct photolysis rate constant, k_{pE} , is given by the equation

$$k_{pE} = 2.30 \sum \phi_{\lambda E} \sigma_{\Delta\lambda} J_{\Delta\lambda} \quad (1)$$

where $\phi_{\lambda E}$ is the quantum yield, $\sigma_{\Delta\lambda}$ is the cross section in the units $\text{cm}^2 \text{ molecule}^{-1}$ averaged over a wavelength interval $\Delta\lambda$, $J_{\Delta\lambda}$ is the actinic solar irradiance in the units $\text{photons cm}^{-2} \text{ day}^{-1}$ averaged over the wavelength interval $\Delta\lambda$, and the summation is taken over the range $\Delta\lambda = 290$ to 800 nm . $J_{\Delta\lambda}$ is the solar actinic irradiance in the atmosphere under clear sky conditions and is a function of latitude and season of the year.

Since this photolysis process is first-order, the half-life, $t_{1/2}$ of a chemical is then given by

$$t_{1/2} = 0.693/k_{pE} \quad (2)$$

A simple first-tier screening test has been developed using equation 1. As an approximation, it is assumed that the quantum yield $\phi_{\lambda E}$ is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in the gas phase is obtained and equation one becomes

$$(k_{pE})_{\max.} = 2.30 \sum \sigma_{\Delta\lambda} J_{\Delta\lambda} \quad (3)$$

Using equation 3 in equation 2, the lower limit for the half-life is then given by

$$(t_{1/2})_{\min.} = 0.693/(k_{pE})_{\max.} \quad (4)$$

The cross section can be determined experimentally by the procedures outlined in Section II and the values of $J_{\Delta\lambda}$ are given in Tables 1 to 3, Section V.A, as a function of latitude and season of the year in the northern hemisphere. These data can then be used in equation 3 to calculate $(k_{pE})_{\max.}$. Finally, $(k_{pE})_{\max.}$ can be substituted in equation 4 to calculate $(t_{1/2})_{\min.}$

D. Applicability and Specificity

This Test Guideline is applicable to all chemicals which have uv-visible absorptions in the range 290-800 nm. Some chemicals only have absorptions below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, dienes, and fluoroalkanes).

This test method is only applicable to pure chemicals and not to the technical grade.

The first-tier screening test can be employed to estimate $(k_{pE})_{\max}$ and $(t_{1/2E})_{\min}$. If these data indicate that gas phase photolysis is an important process relative to other gas phase transformation processes (e.g., oxidation with hydroxyl radicals or ozone), then it is recommended that an upper-tier photolysis test be carried out to determine the quantum yield and thus more precise environmentally relevant rate constants and half-lives in sunlight. The data obtained from this first-tier test method can be used to determine $(k_{pE})_{\max}$ for a test chemical as a function of latitude and season of the year in the northern hemisphere under clear sky conditions. These rate constants are in a form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

II. TEST PROCEDURES

The procedures outlined in this Test Guideline are based on the method proposed by Mill et al. (1982) and developed by Pitts et al. (1981). It is also recommended that Test Guideline

CG-1050, Absorption in Aqueous Solution: Ultraviolet/Visible Spectra, be consulted for additional guidance.

A. Test Conditions

1. Ultraviolet-Visible Spectrophotometer

Although single-beam spectrophotometers may be used, recording double beam spectrophotometers are highly recommended. It is extremely important that the spectrophotometer be able to scan over the wavelength region 270 to 800 nm and have an absorbance sensitivity, at a signal/noise ratio of one, of approximately 0.001. It is important that the spectrophotometer be able to attain a 90% separation of two monochromatic spectral features approximately 4 nm apart, peak to peak (i.e., the resolution should be at least 4 nm). It is also desirable to have a spectrophotometer that can accommodate absorption cells of length ≥ 10 cm. A Cary 219 UV-Visible Spectrophotometer, or an equivalent model, is highly recommended.

2. Vapor and Liquid Absorption Cells

Long pathlength cells are preferable; however, many commercial spectrophotometers will only accept absorption cells of 10 cm or less. A suitable vapor cell is depicted in Figure 1 and can be constructed as follows. The vapor cell should be constructed of Pyrex, 1 cm o.d. and 10 cm in length, and be fitted with plane parallel quartz windows at each end. The quartz windows can be conveniently attached to the Pyrex cell with vacuum tight epoxy resin (e.g., Torr-Seal, Varian Associates) only applied to the outside surface. A Teflon

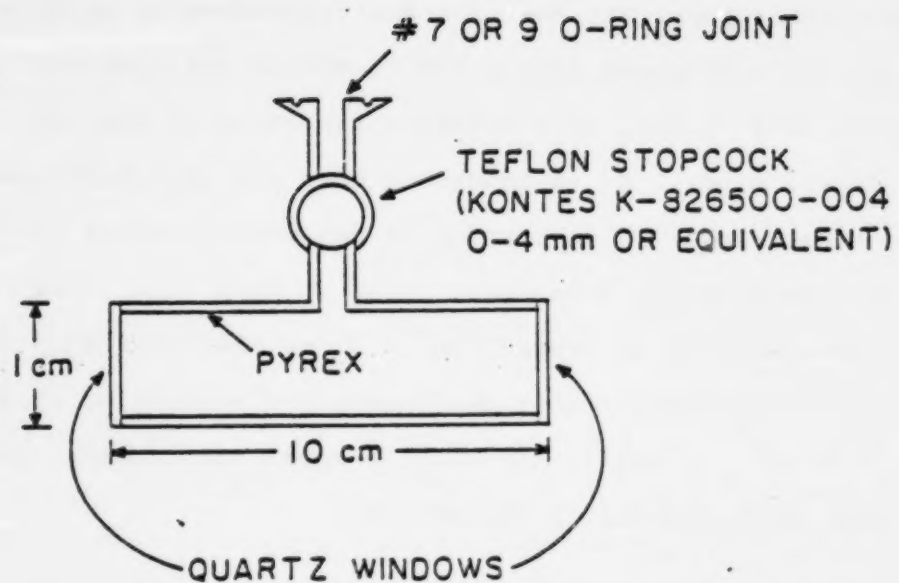


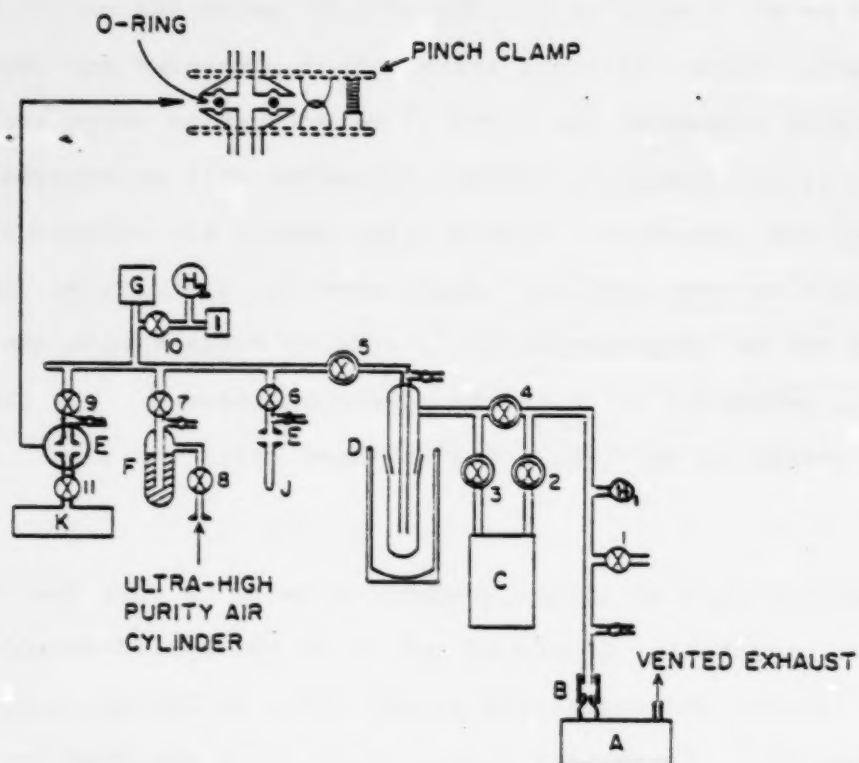
Figure 1. Gas Absorption Cell

stopcock (or a Pyrex "o" ring stopcock) should be connected to the cell and contain an "o"-ring joint. The "o"-ring joint (e.g., #7 or #9, Kontes or Ace Glass) must match the one on the vacuum rack. Viton "o"-rings are highly recommended and should be frequently inspected for signs of deterioration which would result in vacuum leaks. A matched reference cell is extremely useful but not essential. However, the sample and reference cells should be very similar. Small spectral differences between the cells can be compensated for by running a blank with the sample and reference cells in the spectrophotometer. The use of stopcock grease is not required with these cells and should be avoided.

A matched pair of liquid absorption cells is very desirable but is not essential. A pair of quartz uv absorption cells, 10 cm in length, and containing ground glass or Teflon stoppers are recommended. These liquid absorption cells are readily available commercially.

3. Vacuum Gas-Handling System

A suitable gas-handling system is shown diagrammatically in Figure 2 and should be constructed completely with Pyrex glass. The components of the gas-handling system are discussed below. The use of stopcock grease is not required and should be avoided.



- A ROTARY PUMP
- B RUBBER TUBING (THICK WALLED)
- C DIFFUSION PUMP
- D TRAP AT LIQUID NITROGEN TEMPERATURE
- E #7 OR 9 O-RING JOINTS
- F MOLECULAR SIEVE 4A TRAP
- G CAPACITANCE MANOMETER
- H THERMOCOUPLE GAUGE
- I IONIZATION GAUGE
- J LIQUID RESERVOIR
- K GAS ABSORPTION CELL

⊗ 0-4 OR 0-5 mm
STOPCOCKS

⊗ 0-8 OR 0-10 mm
STOPCOCKS

Figure 2. Schematic of Gas Handling Vacuum Rack

a. Vacuum Pumping System

In order to achieve a good vacuum, i.e., pressures $<10^{-5}$ torr (1.3×10^{-6} kPa), two pumps are required. The forepump (A) must be capable of achieving a pressure <0.05 torr (0.0065 kPa). A rotary pump (e.g., a Welch Model 1402 Duo-Seal or an equivalent model) is recommended. The forepump can be attached to the vacuum system by means of heavy-walled rubber vacuum tubing (B) (or any flexible vacuum tubing). The exhaust from this pump should be vented into a hood.

The second pump, a high vacuum model, should be a multi-stage oil diffusion pump (C) [e.g., a Consolidated Vacuum Corp. VMF-10 or VMF-20 or an equivalent model]. The pump fluid should be a silicone oil with a room temperature vapor pressure of $<10^{-6}$ torr (1.3×10^{-7} kPa) [e.g., Dow-Corning D.C. 702 or 703, or an equivalent grade].

It is extremely important that the pumping system contain a trap (D) cooled with liquid nitrogen. The cone and socket joint on this trap can be conveniently sealed with Apiezon W wax, or an equivalent grade. This wax only requires gentle heating to apply and makes an effective vacuum seal. It is possible that a few test chemicals could dissolve Apiezon W wax. In this case, an inert silicone grease may be used to seal the trap.

5. Vacuum Rack

The recommended vacuum rack assembly is depicted in Figure 2. All stopcocks should be of Teflon with Viton "o"-rings [Kontes K-826500 or K-826510 series or equivalent grades (or Pyrex "o" ring stopcocks)]. The "o"-ring joints (E) [#7 or #9] must be compatible with those on the gas absorption cell (K) or on the liquid reservoir (J). These "o"-ring joints should be clamped by pinch clamps with a screw lock device (e.g., Thomas #18A, or an equivalent grade).

c. Pressure Gauges

Three pressure gauges are required:

- (i) An ionization gauge to measure high vacuum [$<10^{-3}$ torr (1.3×10^{-4} kPa)];
- (ii) a thermocouple gauge to monitor the pressure in the range 10^{-3} to 1 torr (1.3×10^{-4} to 0.13 kPa). A convenient pressure monitoring system which contains ionization and thermocouple gauges is a Consolidated Vacuum Corp. Model GIC-300A or an equivalent model; and
- (iii) a pressure gauge to monitor the pressure of the test chemical and diluent in the range 0.01 to 760 torr (0.0013 to 101.3 kPa); for example, an MKS Baratron 310 BHS-1000 with the associated 170-6C electronics unit and a digital readout or an equivalent model. While this vacuum gauge exhibits a slow zero drift,

it can be readily rezeroed using the ionization gauge, i.e., when the ionization gauge reads approximately 10^{-3} torr (0.00013 kPa) or less.

B. Operation of the Gas-Handling System

Since there are a wide variety of procedures available for operating a gas handling system, the method used is left to the discretion of the tester. For those testers who do not have experience in handling a vacuum system, the detailed procedure described in an Appendix, Section V.C, is highly recommended.

C. Preparation of Samples

1. Preparation of the Gas Phase

Test Chemical Sample: Preliminary Steps

If the test chemical is a gas at room temperature, then attach the gas container to the "o"-ring at the point where the liquid reservoir (J) is placed. Close stopcocks 2 and 3 and open 4. Pump until the pressure is less than 10^{-2} torr (1.3×10^{-3} kPa) as read on thermocouple gauge (H_2). Then open stopcocks 2 and 3 and close 4 and pump until the pressure is less than 10^{-5} torr (1.3×10^{-6} kPa) as read on the ionization gauge (I).

If the test chemical is a liquid at room temperature, add a few cm^3 of liquid to a reservoir tube (J), sealed at one end and containing an "o"-ring at the other end, and connect the tube via the "o"-ring to stopcock 6. Freeze the sample with a Dewar

containing liquid nitrogen, close stopcocks 2 and 3 and open 4 and 6. Degas the test chemical by allowing it to warm up to the liquid state, briefly degas, and refreeze the liquid. Repeat this process three or more times until the evolution of gas bubbles cease upon thawing. Freeze the liquid, open stopcocks 2 and 3 and close 4. Pump until the pressure is less than 10^{-5} torr (1.3×10^{-6} kPa) as indicated by the ionization gauge (I). Close stopcock 6.

2. Introduction of the Test Chemical into the Gas Absorption Cell

For introduction of the test chemical into the gas absorption cell, close stopcocks 5, 7 and 10, with 9 and 11 open. If the test chemical is a gas, then stopcock 6 should be opened and the gas container valve is gradually opened to admit the gas into the gas handling manifold and gas absorption cell until the desired pressure is attained, as read on the capacitance manometer (G). Close the gas container valve and stopcock 6 and allow approximately 5 minutes before the final pressure at (G) is read. If the pressure has not stabilized in approximately 5 minutes, allow the cell to condition for several hours before the final pressure at (G) is read.

For a liquid chemical in the reservoir (J), which has been degassed and is at liquid nitrogen temperature, the liquid nitrogen Dewar should be removed and stopcock 6 opened. The cold liquid in the reservoir (J) is allowed to warm up until the required pressure is attained, as read by the capacitance manometer (G). Close stopcock 6 and cool the reservoir again with liquid nitrogen and allow approximately 5 minutes before the final pressure at (G) is read. If the pressure has not stabilized in approximately 5 minutes, allow the cell to condition for several hours before the final pressure at (G) is read.

With stopcocks 6, 8 and 11 closed and 5, 7, 9, and 10 open, the gas handling manifold is evacuated as described previously to a pressure less than 10^{-5} torr (1.3×10^{-6} kPa). Stopcocks 5 and 10 are then closed and ultra-high purity air from a cylinder is admitted into the gas handling manifold via stopcock 8 and through the trap (F) containing Molecular Sieve 4A. When the manifold is at one atmosphere pressure, as measured by pressure gauge (G), stopcock 11 is briefly opened to pressure the gas absorption cell to one atmosphere, and then closed. Stopcocks 8 and 9 are closed and the gas handling system is evacuated as described previously. The gas absorption cell can then be removed from (E) and covered to avoid photolysis.

Based on the pressure P of the test chemical, as measured by gauge (G), the concentration of the gas sample is

$$C \text{ (molecules cm}^{-3}\text{)} = 9.658 \times 10^{18} \frac{P(\text{torr})}{T(^{\circ}\text{K})} \quad (5)$$

$$C \text{ (molecules cm}^{-3}\text{)} = 1.285 \times 10^{18} \frac{P(\text{kPa})}{T(^{\circ}\text{K})} \quad , \quad (5a)$$

where T is the room temperature in $^{\circ}\text{K}$, which should be routinely monitored with a thermometer.

The recommended pressure of the test chemical should be in the range 1-5 torr (0.13 - 0.65 kPa) where the Beer-Lambert law is obeyed. A final check on whether the test chemical obeys the Beer-Lambert law can be accomplished by demonstrating the constancy of the cross section at three partial pressures differing by a factor of 10.

3. Preparation of Solution Phase

Test Chemical Sample

If the properties of the test chemical (i.e., small cross sections, low vapor pressure) are such that the maximum absorbance obtainable is one-tenth of the most sensitive spectrophotometer scale or less (i.e., < 0.001 absorbance), a solution-phase study should be undertaken. The most sensitive scale may be limited by inherent spectrophotometer noise. For example, a given spectrophotometer's most sensitive scale is 0.00 to 0.10 absorbance units. Therefore, a test chemical for which

the product of its maximum cross section and its concentration is less than 0.001 (in a 10 cm cell) could not be analyzed in the vapor phase with this particular spectrophotometer.

The following spectroscopic grade chemicals are recommended to prepare solutions: chloroform, n-hexane, acetonitrile, and cyclohexane. Solutions of up to 10% by volume of test chemical can be prepared in one of these solvents in the standard manner.

The concentration of the test chemical is given by the equations

$$C \text{ (molecules cm}^{-3}\text{)} = 6.023 \times 10^{23} \frac{\text{mass (gms)}}{\text{F.W. (V}_d\text{)}} \quad (6)$$

$$C \text{ (molecules cm}^{-3}\text{)} = 6.023 \times 10^{23} \frac{V_s \rho}{\text{F.W. (V}_d\text{)}} \quad (6a)$$

where V_s is the volume of test chemical delivered into a volume V_d of solvent in cm^3 , F.W. is the formula weight of the test chemical in gms, and ρ is the density of the test chemical in gms cm^{-3} at the room temperature the solution was prepared.

D. Procedure for Obtaining the Spectrum

As a general guide to obtaining uv-visible absorption spectra, the procedures outlined in Test Guideline CG-1050, Absorption in Aqueous Solution: Ultraviolet/Visible Spectra, are highly recommended. Since the method presented in this procedure was developed by Pitts et al. (1981), it is highly recommended that this report be consulted for further details.

1. Determination of the Cell Pathlength

The method for determining the cell pathlength of gas or liquid cells is left to the discretion of the tester. However, the method listed in Test Guideline CG-1050, using one of their reference compounds, is highly recommended.

2. Gas Phase Spectrum

Measure the absorbance of the test chemical in duplicate relative to a matched cell filled with ultra-high purity air from the same cylinder similarly passed through trap (F) containing the molecular sieve. The absorbance should be measured at wavelengths $\lambda > 280$ nm using minimum slit widths. Record, in duplicate, the baseline when both the same and reference cells are filled with high purity air dried through the molecular sieve and at the same settings as used for the test chemical sample. These data will be used to calculate the cross sections, $\sigma'_{\Delta\lambda}$, at the appropriate wavelength intervals listed in Tables 1-3, Section V.A.

3. Solution Phase Spectrum

Measure the absorbance of the test chemical in duplicate relative to a matched cell containing the solvent. The absorbance should be measured for wavelengths $\lambda > 280$ nm using the minimum slit widths. Record, in duplicate, the baseline when both the sample and reference cells are filled with the

solvents. These data will be used to calculate the cross sections, $\sigma'_{\Delta\lambda}$, for the appropriate wavelength intervals listed in Tables 1-3, Section V.A.

The concentration of the test chemical should be in the range where the Beer-Lambert law is obeyed. A check on whether the test chemical obeys this law can be accomplished by demonstrating the constancy of the cross section at three concentrations differing by a factor of 10.

III. DATA AND REPORTING

A. Treatment of Results

1. Determination of the Cross Section from the Gas Phase Spectrum

The cross section, $\sigma'_{\Delta\lambda}$, can be determined from the gas phase absorption spectrum and the Beer-Lambert law in the form

$$\sigma'_{\Delta\lambda} = \frac{A_{\Delta\lambda}}{C\ell} \quad (7)$$

where $A_{\Delta\lambda}$ is the absorbance in the wavelength interval $\Delta\lambda$, C is the concentration of test chemical in molecules cm^{-3} , and ℓ is the cell pathlength in cm. The cross section of the test chemical should be determined for the wavelength intervals listed in Tables 1-3, Section V.A.

There are at least three nondestructive methods of determining the absorbance over a specified wavelength interval: estimation, square counting, and planimetry. For many spectra, estimating an average absorbance over a small wavelength interval is sufficient to yield accurate results. However, for spectra containing rapidly changing absorptions and complex fine structure, square counting or planimetry should be used. These two methods require the integration of a definite region (in $A_\lambda \times \text{nm}$) followed by division by the width of the region in nm to obtain the absorbance. The method using a compensating polar planimeter is the most accurate and is highly recommended. The absorbance should be obtained from the average of three tracings.

2. Determination of the Cross Section from the Solution Phase Spectrum

The cross section, $\sigma_{\Delta\lambda}$, can be determined from the solution phase spectrum using equation 7 for the wavelength intervals listed in Tables 1-3, Section V.A. For solution spectra, estimating an average absorbance over the wavelength intervals is sufficient to yield accurate results.

3. Estimation of the Maximum Direct Photolysis
Rate Constant and Minimum Half-Life
in the Gas Phase

Using the cross sections obtained from the spectra and the values of $J_{\Delta\lambda}$ from Tables 1-3, Section V.A, the maximum direct photolysis rate constant $(k_{pE})_{\max}$. can be calculated at a specific latitude and season of the year using equation 3. The minimum half-life, $(t_{1/2})_{\min.}$, can be calculated using this $(k_{pE})_{\max}$. in equation 4.

An example is presented in an Appendix, Section V.B, to illustrate how the test data obtained in this Test Guideline can be used.

B. Test Data Report

- (1) Submit the original chart, or photocopy, containing a plot of absorbance vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10 nm intervals. Each spectrum should be clearly marked.
- (2) Gas Phase Spectra
 - (a) Report the pressure of the test chemical in torr (or kPa), the concentration in molecules cm^{-3} , and the pathlength of the sample cell in cm. Describe the method used to determine the pathlength and report the experimental data.

- (b) Report the wavelength interval for each 10 nm over the region of absorption, the value of the absorbance ($A_{\Delta\lambda}$) for each replicate, the mean absorbance, and the mean cross section in $\text{cm}^2 \text{molecule}^{-1}$.
- (c) Report the estimated maximum direct photolysis rate constant in days^{-1} and the corresponding minimum half-life in days at 10° , 30° , and 50° north latitude for the summer and winter solstices.

(3) Solution Phase Spectra

- (a) Report the concentration of the test chemical in molecules cm^{-3} , the type of cell used (quartz or borosilicate), and the pathlength in cm. Describe the method used to determine the pathlength and report the experimental results.
- (b) Report the identity of the solvent.
- (c) Report the wavelength interval over the region of absorption, the value of the absorbance ($A_{\Delta\lambda}$) of each replicate, the mean absorbance, and the mean cross section ($\sigma'_{\Delta\lambda}$) in $\text{cm}^2 \text{molecule}^{-1}$.
- (d) Report the estimated maximum direct photolysis rate constant in days^{-1} and the corresponding minimum half-life in days at 10° , 30° , and 50° north latitude for the summer and winter solstices.

- (4) Report the name, structure, and purity of the test chemical.
- (5) Submit a recent spectrum on appropriate reference chemicals for photometric and wavelength accuracy.
- (6) Report the name and model of the spectrophotometer used.
- (7) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, given, etc.
- (8) Report anything unusual about the test; e.g., if the Beer-Lambert law is not obeyed at a pressure of 1-5 torr (0.13 to 0.65 kPa), report the pressure at which the deviation was overcome and the experimental data. If the Beer-Lambert law is not obeyed in solution at high concentrations, report the concentration at which the deviation was overcome and the experimental data.
- (9) Report any other relevant information.

IV. REFERENCES

Mill T, Mabey WR, Bomberger DC, Chou T-W, Hendry DG, and Smith JH. 1982. Laboratory protocols for evaluating the fate of organic chemicals in air and water. Chapter 5. EPA 600/3-82-022.

Pitts JN Jr., Winer AM, Fitz DR, Knudsen AK, and Atkinson R. 1981. Experimental protocol for determining absorption cross sections of organic chemicals. EPA 600/3-81-051.

V. APPENDICIESA. Appendix A. Tables 1-3 [from Mill et al. (1982)]TABLE 1. $J_{\Delta\lambda}$ (photons $\text{cm}^{-2} \text{ day}^{-1}$) VALUES AT 10°N LATITUDE^a

Wavelength Range, nm	Solstice				Equinox	Season Average				
	Summer		Winter			Spring/Summer		Fall/Winter		
285-295	0		0		0		0		0	
295-305	6.39	17	3.31	17	6.38	17	6.63	17	4.6	17
305-315	9.701	18	6.679	18	9.522	18	9.815	18	7.91	18
315-325	2.451	19	1.852	19	2.386	19	2.457	19	2.685	19
325-335	4.474	19	3.518	19	4.341	19	4.468	19	3.877	19
335-345	4.945	19	3.957	19	4.789	19	4.828	19	4.321	19
345-355	5.666	19	4.605	19	5.479	19	5.638	19	4.735	19
355-365	5.85	19	4.758	19	5.656	19	5.820	19	4.925	19
365-375	7.301	19	5.976	19	7.655	19	7.226	19	6.357	19
375-385	7.157	19	5.891	19	6.913	19	7.125	19	6.343	19
385-395	7.329	19	6.065	19	7.075	19	7.278	19	6.663	19
395-405	1.004	20	8.341	19	9.687	19	9.966	19	8.927	19
405-415	1.275	20	1.063	20	1.23	20	1.265	20	1.135	20
415-425	1.331	20	1.114	20	1.283	20	1.320	20	1.187	20
425-435	1.349	20	1.133	20	1.30	20	1.338	20	1.205	20
435-445	1.491	20	1.256	20	1.436	20	1.478	20	1.339	20

TABLE 1. Continued

Wavelength Range, nm	Solstice				Equinox	Season Average				
	Summer		Winter			Spring/Summer		Fall/Winter		
445-455	1.718	20	1.449	20	1.655	20	1.702	20	1.538	20
455-465	1.857	20	1.568	20	1.789	20	1.840	20	1.664	20
465-475	1.914	20	1.619	20	1.843	20	1.896	20	1.716	20
475-485	1.941	20	1.645	20	1.869	20	1.922	20	1.742	20
485-495	1.945	20	1.652	20	1.873	20	1.926	20	1.747	20
495-515	3.970	20	3.370	20	3.823	20	3.932	20	3.565	20
515-535	4.013	20	3.41	20	3.863	20	3.974	20	3.605	20
535-555	3.995	20	3.398	20	3.846	20	3.956	20	3.591	20
555-575	4.053	20	3.446	20	3.902	20	4.014	20	3.692	20
575-595	4.197	20	3.573	20	4.040	20	3.659	20	3.774	20
595-635	8.529	20	7.235	20	8.211	20	8.446	20	7.652	20
635-675	8.976	20	7.658	20	8.637	20	8.884	20	8.077	20
675-715	9.077	20	7.778	20	8.732	20	8.961	20	8.186	20
715-755	8.744	20	7.514	20	8.410	20	8.650	20	7.897	20
755-795	8.460	20	7.287	20	8.135	20	8.367	20	7.648	20

^aSecond number in column is the power of ten by which the first number is multiplied.

TABLE 2. $J_{\Delta\lambda}$ (photons $\text{cm}^{-2} \text{ day}^{-1}$) VALUES AT 30°N LATITUDE^a

Wavelength Range, nm	Solstice				Equinox	Season Average				
	Summer		Winter			Spring/Summer		Fall/Winter		
285-295	1.0	16	0		0		1.18	15	0	
295-305	7.40	17	6.80	16	3.98	17	6.24	17	1.78	17
305-315	1.09	19	2.83	18	7.49	18	9.77	18	4.60	18
315-325	2.74	19	1.00	19	2.03	19	2.50	19	1.41	19
325-335	4.98	19	2.11	19	3.82	19	4.58	19	2.79	19
335-345	5.49	19	2.48	19	4.28	19	5.07	19	3.20	19
345-355	6.28	19	3.11	19	4.96	19	5.82	19	3.68	19
355-365	6.49	19	3.10	19	5.13	19	6.01	19	3.92	19
365-375	8.09	19	3.95	19	6.43	19	7.51	19	4.96	19
375-385	7.93	19	3.95	19	6.33	19	7.36	19	4.92	19
385-395	8.12	19	4.12	19	6.51	19	7.55	19	5.09	19
395-405	1.11	20	5.72	19	8.94	19	1.03	20	7.04	19
405-415	1.41	20	7.36	19	1.14	20	1.31	20	8.64	19
415-425	1.47	20	7.80	19	1.19	20	1.37	20	9.37	19
425-435	1.49	20	7.99	19	1.21	20	1.39	20	9.67	19
435-445	1.65	20	8.93	19	1.34	20	1.54	20	1.08	20

TABLE 2. Continued

Wavelength Range, nm	Solstice				Equinox	Season Average				
	Summer		Winter			Spring/Summer		Fall/Winter		
445-455	1.90	20	1.04	20	1.55	20	1.77	20	1.25	20
455-465	2.05	20	1.13	20	1.67	20	1.92	20	1.35	20
465-475	2.12	20	1.17	20	1.73	20	1.98	20	1.40	20
475-485	2.15	20	1.19	20	1.75	20	2.01	20	1.42	20
485-495	2.15	20	1.20	20	1.76	20	2.01	20	1.43	20
495-515	4.39	20	2.45	20	3.59	20	4.10	20	2.92	20
515-535	4.44	20	2.49	20	3.63	20	4.15	20	2.96	20
535-555	4.42	20	2.49	20	3.62	20	4.13	20	2.95	20
555-575	4.48	20	2.52	20	3.67	20	4.19	20	2.99	20
575-595	4.64	20	2.62	20	3.80	20	4.34	20	3.11	20
595-635	9.44	20	5.39	20	7.70	20	8.81	20	6.34	20
635-675	9.92	20	5.72	20	8.15	20	9.28	20	6.72	20
675-715	1.00	21	5.85	20	8.27	20	9.39	20	6.84	20
715-755	9.67	20	5.70	20	7.98	20	9.05	20	6.64	20
755-795	9.35	20	5.57	20	7.74	20	8.76	20	6.46	20

^aSecond number in column is the power of ten by which the first number is multiplied.

TABLE 3. $J_{\Delta\lambda}$ (photons $\text{cm}^{-2} \text{ day}^{-1}$) VALUES AT 50°N LATITUDE^a

Wavelength Range, nm	Solstice				Equinox				Season Average			
	Summer		Winter						Spring/Summer		Fall/Winter	
285-295	0		0		0		0		0		0	
295-305	5.7	17	6.0	14	1.12	17	3.763	17	2.55	16		
305-315	1.005	19	2.981	17	3.945	18	7.724	18	1.398	18		
315-325	2.680	19	1.857	18	1.317	19	2.173	19	5.699	18		
325-335	5.0	19	5.429	18	2.714	19	4.162	19	1.314	19		
335-345	5.577	19	7.118	18	3.163	19	4.700	19	1.597	19		
345-355	6.446	19	8.513	18	3.86	19	5.495	19	1.984	19		
355-365	6.659	19	9.536	18	3.923	19	5.670	19	2.057	19		
365-375	8.341	19	1.248	19	4.985	19	7.130	19	2.648	19		
375-385	8.204	19	1.279	19	4.967	19	7.038	19	2.646	19		
385-395	8.427	19	1.070	19	5.165	19	7.255	19	2.816	19		
395-405	1.157	20	1.951	19	7.161	19	9.987	19	3.949	19		
405-415	1.472	20	2.569	19	9.194	19	1.274	20	5.132	19		
415-425	1.540	20	2.792	19	9.715	19	1.337	20	5.478	19		
425-435	1.564	20	2.915	19	9.938	19	1.361	20	5.652	19		
435-445	1.732	20	3.326	19	1.109	20	1.510	20	6.366	19		

TABLE 3. Continued

Wavelength Range, nm	Solstice				Equinox	Season Average				
	Summer		Winter			Spring/Summer		Fall/Winter		
445-455	1.996	20	3.91	19	1.283	19	1.743	20	7.413	19
455-465	2.159	20	4.30	19	1.392	20	1.886	20	8.087	19
465-475	2.227	20	4.533	19	1.443	20	1.948	20	8.444	19
475-485	2.261	20	4.685	19	1.471	20	1.981	20	8.659	19
485-495	2.268	20	4.769	19	1.483	20	1.983	20	8.758	19
495-515	4.629	20	9.789	19	3.026	20	4.060	20	1.791	20
515-535	4.682	20	1.001	20	3.069	20	4.109	20	1.823	20
535-555	4.664	20	1.002	20	3.062	20	4.095	20	1.822	20
555-575	4.732	20	1.014	20	3.104	20	4.153	20	1.845	20
575-595	4.903	20	1.063	20	3.228	20	4.308	20	1.926	20
595-635	9.935	20	2.227	20	6.631	20	8.749	20	3.983	20
635-675	1.048	20	2.463	20	7.015	20	9.253	20	3.428	20
675-715	1.061	21	2.610	20	7.165	20	9.40	20	4.431	20
715-755	1.023	21	2.644	20	6.977	20	9.089	20	4.370	20
755-795	9.902	20	2.648	20	6.809	20	8.821	20	4.311	20

^aSecond number in column is the power of ten by which the first number is multiplied.

B. Appendix B. Illustrative Example*

Consider a chemical plant located in Freeport, Texas, which produces acrolein [$\text{CH}_2 = \text{CHCHO}$] continuously every day of the year. Despite the fact that all acrolein wastes, including vented vapors, are treated in a waste treatment plant, some acrolein escapes into the atmosphere. The chemical plant is located at 29° north latitude. Estimate the maximum sunlight direct photolysis rate constant and the corresponding minimum half-life in the atmosphere in the vicinity of the plant for the winter and summer season solstices under clear sky conditions.

The vapor phase spectrum of acrolein was obtained by the procedure outlined in this Test Guideline and is depicted in Figure 3.* The pathlength of the sample gas absorption cell was measured according to the recommended procedure and was found to be 9.98 cm. The gas absorption cell contained 6.52×10^{16} molecules cm^{-3} of acrolein. A compensating polar planimeter was used to integrate each 10 nm interval throughout the region of absorption from 285 nm to 425 nm in both the sample and blank spectra. Based on triplicate measurements, one square, corresponding to 0.001 absorbance units (A), was found to be 0.148 vernier units (v.u.). The mean absorbance ($A_{\Delta\lambda}$) was obtained from these spectra and the mean cross section ($\sigma'_{\Delta\lambda}$) was obtained using equation 7 for each wavelength interval. All the results are summarized in Table 4.

*The spectral data were taken from the work of Pitts et al. (1981).

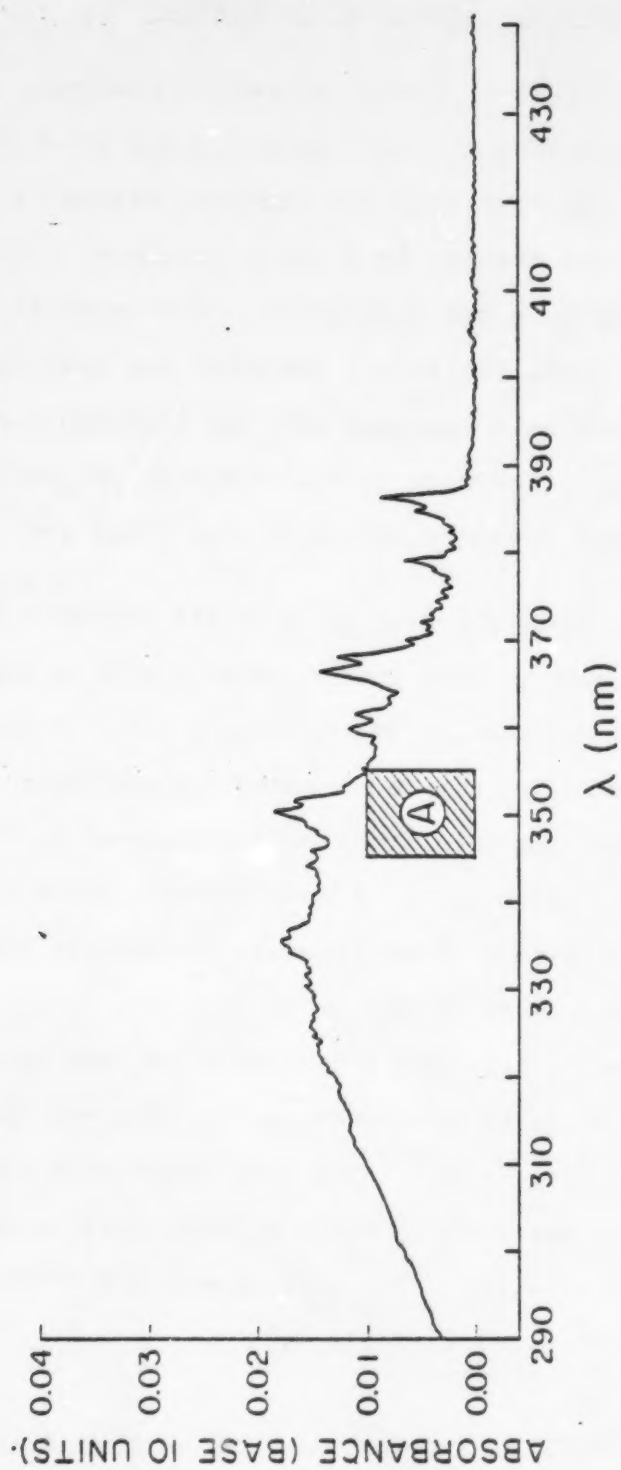


Figure 3. Gas Phase Absorption Spectrum of Acrolein

TABLE 4. ABSORBANCE AND CROSS SECTION FOR ACROLEIN VAPOR*

Wavelength Interval (nm)	Mean Absorbance [$A_{\Delta\lambda}$]	Mean Cross Section [$\sigma_{\Delta\lambda}$] ($\text{cm}^2 \text{ molecule}^{-1}$)
285 - 295	0.0037	5.69×10^{-21}
295 - 305	0.0066	1.01×10^{-20}
305 - 315	0.0104	1.60×10^{-20}
315 - 325	0.0137	2.11×10^{-20}
325 - 335	0.0156	2.40×10^{-20}
335 - 345	0.0156	2.40×10^{-20}
345 - 355	0.0151	2.32×10^{-20}
355 - 365	0.0096	1.48×10^{-20}
365 - 375	0.0073	1.12×10^{-20}
375 - 385	0.0031	4.76×10^{-21}
385 - 395	0.0016	2.46×10^{-21}
395 - 405	0.0004	6.15×10^{-22}
405 - 415	0.0003	4.61×10^{-22}
415 - 425	0.0000	0.00

* $6.52 \times 10^{16} \text{ molecules cm}^{-3}$ in a 9.98 cm gas absorption cell.

A sample calculation is given for the wavelength interval 345-355 nm. For convenience, the area A, corresponding to 100 squares was blocked off in this absorption area and was not integrated with the planimeter. The average vernier reading of the remaining absorption area was 7.2 v.u. Hence,

$$\frac{7.2 \text{ v.u.}}{0.148 \text{ v.u./square}} = 49 \text{ squares,}$$

and the total area in the spectrum in the wavelength interval 345-355 nm is 149 squares. This number of squares corresponds to 0.0149 absorbance units:

$$\frac{(149 \text{ squares})(0.001 \text{ A/square})}{10} = 0.0149 \text{ A.}$$

From the blank spectrum, the baseline absorbance ($A_{\Delta\lambda \text{ blank}}$) over this interval was -0.0001. The sample trace lay at -0.0001 absorbance units relative to a zero point at 450 nm. The observed sample absorbance is then equal to 0.0150 (0.0149 + 0.0001). The absolute corrected absorbance for the sample is given by

$$A_{\Delta\lambda \text{ sample}}^{\text{corr.}} = A_{\Delta\lambda \text{ sample}}^{\text{obs.}} - A_{\Delta\lambda \text{ blank}}$$

$$A_{\Delta\lambda \text{ sample}}^{\text{corr.}} = 0.0150 - (-0.001) = 0.0151 \text{ A.}$$

Using equation 7 and the values for the corrected sample absorbance, ℓ , and C, the mean cross section for the wavelength interval 345-355 nm is

$$\sigma'_{\Delta\lambda} = \frac{0.0151}{6.52 \times 10^{16} \text{ molecules cm}^{-3} (9.98 \text{ cm})}$$

$$\sigma'_{\Delta\lambda} = 2.3 \times 10^{-20} \text{ cm}^2 \text{ molecule}^{-1}.$$

Since the plant is located at 29° north latitude, the closest $J_{\Delta\lambda}$ values are at 30° north latitude. These values are obtained from Table 2 and are summarized in Table 5 for the summer and winter season solstices. Using the data in Tables 4 and 5, the products $\sigma'_{\Delta\lambda} J_{\Delta\lambda}$ are calculated for each wavelength interval and the results are summarized in Table 5 for each of the solstices. The terms $\sum \sigma'_{\Delta\lambda} J_{\Delta\lambda}$ are also summarized for each solstice at the bottom of Table 5. Using these data in equations 3 and 4 yields:

<u>Summer</u>	<u>Winter</u>
$(k_{pE})_{\text{max.}} = 16.9 \text{ days}^{-1}$	$(k_{pE})_{\text{max.}} = 7.64 \text{ days}^{-1}$
$(t_{1/2})_{\text{min.}} = 0.041 \text{ days}$	$(t_{1/2})_{\text{max.}} = 0.091 \text{ days}$

Thus, arolein transforms rapidly under clear sky conditions in the vicinity of the plant at Freeport, Texas on the summer and winter season solstices.

TABLE 5. CALCULATION OF $k_p^{\max}$. FOR ACROLEIN VAPOR -
DAY RATE AT 30°N ON WINTER AND SUMMER SOLSTICES

Wavelength Interval (nm)	Summer Solstice		Winter Solstice	
	$J_{\Delta\lambda}$ (photons $\text{cm}^{-2} \text{ day}^{-1}$)	$\sigma'_{\Delta\lambda} J_{\Delta\lambda}$ (day $^{-1}$)	$J_{\Delta\lambda}$ (photons $\text{cm}^{-2} \text{ day}^{-1}$)	$\sigma'_{\Delta\lambda} J_{\Delta\lambda}$ (day $^{-1}$)
285 - 295	1.0×10^{16}	0.00006	0.00	0.000
295 - 305	7.40×10^{17}	0.008	6.80×10^{16}	0.001
305 - 315	1.09×10^{19}	0.174	2.83×10^{18}	0.025
315 - 325	2.74×10^{19}	0.578	1.00×10^{19}	0.211
325 - 335	4.98×10^{19}	1.200	2.11×10^{19}	0.506
335 - 345	5.49×10^{19}	1.360	2.48×10^{19}	0.595
345 - 355	6.28×10^{19}	1.460	3.11×10^{19}	0.722
355 - 365	6.49×10^{19}	0.961	3.10×10^{19}	0.459
365 - 375	8.09×10^{19}	0.906	3.95×10^{19}	0.442
375 - 385	7.93×10^{19}	0.378	3.95×10^{19}	0.188
385 - 395	8.12×10^{19}	0.200	4.12×10^{19}	0.101
395 - 405	1.11×10^{20}	0.068	5.72×10^{19}	0.035
405 - 415	1.41×10^{20}	0.065	7.36×10^{19}	0.034
415 - 425	1.47×10^{20}	0.000	7.80×10^{19}	0.000
$\sum \sigma'_{\Delta\lambda} J_{\Delta\lambda} = 7.36_4$			$\sum \sigma'_{\Delta\lambda} J_{\Delta\lambda} = 3.31_9$	

C. Appendix C. Operation of the Gas Handling System

The following procedure briefly describes the recommended typical and detailed operation of a gas handling system.

- (1) Close all stopcocks and turn on the rotary pump (A). Open stopcock 4 and place a Dewar containing liquid nitrogen around trap (D). Measure the pressure with the thermocouple gauge H_1 . When the pressure is less than 0.1 torr (0.013 kPa) open stopcocks 5 and 10, pump out this portion of the manifold, and measure the pressure with the thermocouple gauge H_2 . When the pressure falls below 10^{-2} torr (1.3×10^{-3} kPa), open stopcock 7 and evacuate F containing activated Linde Molecular Sieve 4A or an equivalent grade. Heat F to approximately 150°C for 1-2 hours under vacuum until the pressure falls to less than 10^{-2} torr (1.3×10^{-3} kPa) as measured on thermocouple gauge H_2 . Open stopcocks 6, 9 and 11 and pump until H_2 falls below 10^{-2} torr (1.3×10^{-3} kPa).
- (2) Turn on the diffusion pump (C) and when this pump has reached operating temperature, open stopcocks 2 and 3 and close stopcock 4. Pump on the manifold until the pressure is $< 10^{-5}$ torr (1.3×10^{-6} kPa) as measured by the ionization gauge (I) and zero on the capacitance manometer (G). It should be noted that the ionization gauge (I) should only be used when H_2 indicates a pressure less than 10^{-2} torr (1.3×10^{-3} kPa).

- (3) It is good practice, after the gas phase spectrum has been obtained, to evacuate the gas absorption cell (K) and the trap (F) prior to shutting down the gas handling system. The gas handling system can be shut down by the following procedure: (a) closing stopcocks 5 to 11, (b) switching off the diffusion pump, (c) when the diffusion pump is cool, closing stopcocks 2 and 3 and opening 4, (d) removing the Dewar from trap (D) and allowing it to warm up, (e) then closing stopcock 4 and switching off the rotary pump and (f) opening stopcock 1 to admit air to the rotary pump, thus preventing suck-back of the rotary pump oil. With this procedure, the vacuum manifold, the trap D and the diffusion pump are left under vacuum. The method of cleaning the liquid reservoir (J) is left to the discretion of the tester. However, as a final step it should be cleaned with reagent grade methanol or dichloromethane as solvent and dried. It is then ready for use.
- In operating a vacuum system with the diffusion pump working, do not expose the diffusion pump to pressures > 0.1 torr of air (1.3×10^{-2} kPa) to avoid the degradation of the pump oil.

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GAS PHASE ABSORPTION CROSS SECTION AND SUNLIGHT PHOTOLYSIS

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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GAS PHASE ABSORPTION CROSS SECTION AND SUNLIGHT PHOTOLYSISI. NEED FOR THE TEST

Numerous chemicals are emitted into the troposphere through man's activities and may be removed by wet or dry deposition or they may be transformed by several reaction pathways. Laboratory and environmental chamber studies have shown that for the troposphere, the following gas phase transformation routes are likely to be important for many chemicals [Finlayson-Pitts and Pitts (1977), Herron et al. (1979), Hendry and Kenley (1979), and Demerjian et al. (1980) and the references therein]:

- (1) Photolysis, which involves the absorption of sunlight, followed by transformation.
- (2) Oxidation with Ozone (O_3).
- (3) Reaction with hydroxyl radicals (OH).

Hence, in order to assess the atmospheric life time of chemicals with respect to gas phase removal processes and the relative importance of each of these reaction pathways, direct photolysis rate constants and/or rate constants for oxidation via OH or O_3 must be experimentally determined for individual chemicals.

When radiation is incident upon a gaseous chemical substance, absorption occurs if the frequency of the radiation corresponds to the energy required for the system to make an allowed transition to a higher energy state. In the case of

sunlight ($\lambda > 290$ nm), the absorption of near uv and visible light results in transitions of valence state electrons. In certain instances, an electronic transition is followed by a gaseous reaction which transforms the parent chemical into one or more products. A cost-effective photolysis test is needed to assess quantitatively the gas phase transformation of chemicals in the troposphere in sunlight. Utilization of this test method will provide a basis for evaluating the possible relative importance of photolysis as one reaction pathway for gaseous chemicals in the troposphere.

II. SCIENTIFIC ASPECTS

A. Development of the Test Method

1. Theoretical Aspects

The sun is the driving force for atmospheric photochemical reactions. In a polluted urban atmosphere, chemical species are produced and destroyed by a complex process that involves numerous specific kinetic reactions. Important chemical species such as NO_2 , HNO_3 , and organic chemicals absorb sunlight in specific wavelength bands and are consequently dissociated. Clearly, this process and the resultant chemical products are strongly dependent on the nature of the incident solar radiation.

The troposphere receives ultraviolet and visible radiation during the day directly from the sun, by scattered light from the sky, and by reflection from the earth's surface. The quantity of radiation received in the troposphere is dependent on the solar irradiance outside the atmosphere, the solar zenith angle, scattering, diffusion, absorption by the atmosphere, and the reflection of sunlight from the surface of the earth. Furthermore, the amount of radiation absorbed by chemical species within the polluted layer in the troposphere depends on the radiation received, the absorption coefficient and the concentration of the chemical species, and on the pathlength. Leighton carried out considerable research in the 1950's on the photochemical processes that occur in the atmosphere, many of his computational results were first reported in 1956 [Leighton and Perkins (1956)], and much of this work was published in a book in 1961 [Leighton (1961)]. His work describes in detail a simple, yet effective, radiative transfer model to calculate the actinic flux at the earth's surface.

Leighton (1961) has shown that for weak absorbance of chemicals in the atmosphere, the average rate of absorption of sunlight per unit volume is

$$I_{a\lambda} = 2.30 \alpha_{\lambda} j^{-1} C [I_{d\lambda} \secant(z) + i I_{s\lambda}] \quad , \quad (1)$$

where $I_{a\lambda}$ is the average rate of absorption of sunlight in photons $\text{cm}^{-3} \text{sec}^{-1}$, $I_{d\lambda}$ and $I_{s\lambda}$ are the direct and sky sunlight intensity in photons $\text{cm}^{-2} \text{sec}^{-1}$, z is the solar zenith angle, C

is the concentration of chemical, α_λ is the decadic absorption coefficient of the chemical, j is a constant which converts the intensity units into units that are compatible with α_λ and C , and i is a constant (i.e., $\bar{L}_s = ih$, where $\bar{L}_s$ is the average pathlength of sky radiation traversing a surface layer of the atmosphere of height h). Leighton indicated that to a good approximation $i = 2$. Leighton called the term in the brackets of equation one J_λ , the actinic irradiance, and equation one becomes

$$I_{a\lambda} = 2.30 \alpha_\lambda j^{-1} C J_\lambda, \quad (2)$$

where

$$J_\lambda = [I_{d\lambda} \secant(z) + i I_{s\lambda}] \quad (3)$$

Letting

$$\sigma'_\lambda = \alpha_\lambda j^{-1}, \text{ equation 2 becomes}$$

$$I_{a\lambda} = 2.30 \sigma'_\lambda C J_\lambda, \quad (4)$$

where σ'_λ is the cross section of a chemical in $\text{cm}^2 \text{ molecule}^{-1}$, C is the concentration of chemical in molecules cm^{-3} , $j = 1$, and J_λ is in $\text{photons cm}^{-2} \text{ sec}^{-1}$. The rate of absorption of sunlight by the chemical over the entire solar region (290 - 800 nm) is

$$I_a = \int I_{a\lambda} d\lambda = 2.30 \int \sigma'_\lambda C J_\lambda d\lambda \quad (5)$$

Since the solar flux is conveniently tabulated as average values of $J_{\Delta\lambda}$ over 10 nm intervals, the integral in equation 5 can be replaced by a summation to yield

$$I_a = 2.30C \sum \sigma'_{\Delta\lambda} J_{\Delta\lambda} \quad , \quad (6)$$

where $\sigma'_{\Delta\lambda}$ represents the absorption cross section averaged over 10 nm intervals.

Using the first and second laws of photochemistry (i.e., the Grotthus-Draper and the Stark-Einstein laws, respectively), the kinetic expression for the rate of disappearance of chemical with time is

$$-\frac{dC}{dt} = 2.30C \sum \phi_{\lambda E} \sigma'_{\Delta\lambda} J_{\Delta\lambda} = k_{pE} C \quad , \quad (7)$$

where $\phi_{\lambda E}$ is the quantum yield and k_{pE} , defined by equation 8, is the direct photolysis rate constant in sunlight and is given by the relationship

$$k_{pE} = 2.30 \sum \phi_{\lambda E} \sigma'_{\Delta\lambda} J_{\Delta\lambda} \quad . \quad (8)$$

Integrating equation 7 yields

$$\ln(C_0/C_t) = k_{pE} t \quad , \quad (9)$$

where C_0 is the initial concentration of the chemical at $t = 0$ and C_t is the concentration of chemical at time t . Equation 9 can be solved for the condition $C_t = C_0/2$ and the half-life of the chemical is then

$$t_{1/2} = 0.693/k_{PE} \quad (10)$$

Leighton's model and actinic flux values ($J_{\Delta\lambda}$) have been a cornerstone in the development of atmospheric chemistry [Leighton and Perkins (1956), Leighton (1961)]. His actinic flux values have been used to determine photodissociation rate constants of several species, especially NO_2 , as a function of time of day or zenith angle. These rate constants have been used in mathematical diffusion modeling of photochemical pollution (Reynolds (1973)) and in the computer simulation of mixtures of reactive chemical species to evaluate various mechanisms for photochemical smog formulation [Demerjian (1974)]. Peterson updated the work of Leighton by using an improved radiative transfer model and tabulated the improved actinic flux values [Peterson (1976)]. Rate constants have been calculated for NO_2 , O_3 , HONO , HONO_2 , CH_2O , CH_3CHO , and H_2O using the updated flux values of Peterson [Scherer and Demerjian (1977)].

2. Test Method

The test method was initially proposed by Hendry in 1979 and was published in 1982 [Mill et al. (1982)]. As a simplification, Hendry assumed that $\phi_{\lambda E} = 1^*$ and thus an upper limit for the gas phase direct photolysis rate constant is obtained. With this assumption, equation 8 becomes

$$(k_{pE})_{\max.} = 2.30 \sum \sigma_{\Delta\lambda}' J_{\Delta\lambda} \quad (11)$$

Substitution of the maximum rate constant in equation 10 yields the minimum half-life

$$(t_{1/2})_{\min.} = 0.693 / (k_{pE})_{\max.} \quad (12)$$

$(k_{pE})_{\max.}$ can be estimated from a knowledge of $\sigma_{\Delta\lambda}'$ and $J_{\Delta\lambda}$ in equation 11 and the minimum half-life can then be estimated by substituting this result in equation 12.

The average cross section for a gaseous molecule can be determined experimentally from a uv-visible absorption spectrum and the values of $J_{\Delta\lambda}$ have been computed by Hendry for 31

* For pollutants in the atmosphere at very low concentrations, the maximum value of the quantum yield is one.

wavelength intervals over the range 290 - 800 nm for the winter and summer solstices and the equinox at 10°, 30°, and 50° north latitude.

a. Actinic Solar Irradiance

To determine the actinic solar irradiance values ($J_{\Delta\lambda}$), it is necessary to know the solar intensity at the top of the atmosphere, the absorption due to atmospheric ozone and aerosols, the molecular scattering (i.e., Rayleigh scattering), and the aerosol scattering (i.e., Mie scattering). Leighton originally estimated $J_{\Delta\lambda}$ values based on limited information [Leighton and Perkins (1956), Leighton (1961)]. Peterson (1976, 1977) reanalyzed the problem using an improved radiative transfer model and more recent data and has calculated better values of $J_{\Delta\lambda}$.

Using the values of $J_{\Delta\lambda}$ from Peterson's work in equation 11, instantaneous rate constants are obtained. Because many chemicals require more than one day to photolyze, Hendry modified the above approach as follows. Schere and Demerjian (1977) published a computer routine to carry out the summation over 10 nm wavelength intervals. This routine calculates instantaneous rate constants throughout the day for any day of the year and any latitude and longitude. The $J_{\Delta\lambda}$ values of Peterson were used and the outputs from the Schere and Demerjian routine were integrated over the entire day to obtain rates on a basis of a 24 hour day. To facilitate the computation of photochemical rate constants, Hendry prepared Tables 1 to 3,

Section V.A of the Test Guideline, which contain day-integrated light intensities $J_{\Delta\lambda}$ as a function of wavelength and latitudes 10° , 30° , and 50° north for the winter and summer solstices and the equinox. In addition, these tables include averaged spring-summer and fall-winter light intensity values. These light intensities refer to $J_{\Delta\lambda}$ values which represent the total available actinic flux in a given wavelength region for the winter and summer solstices at a specific latitude. The wavelength ranges in Tables 1 to 3 are divided into 31 intervals over the total range 290 - 800 nm to maintain reliability of the calculations and yet allow them to be carried out rapidly.

b. Determination of the Cross Section

The cross section of a test chemical can be obtained from the Beer-Lambert law and the ultraviolet-visible absorption spectrum. The Beer-Lambert law states that for an absorbing chemical present in the gas phase, the decrease in intensity of light with thickness $(-dI/d\ell)$, at a fixed wavelength λ , is proportional to the intensity I in photons $\text{cm}^{-2} \text{ sec}^{-1}$ and the concentration of the chemical C in the gas phase [Prutton and Maron (1951)]. Therefore,

$$-\frac{dI}{d\ell} = \sigma_{\lambda} CI \quad , \quad (13)$$

where σ_{λ} is the proportionality constant. Equation 13 can be easily integrated to give the Beer-Lambert equation

$$\ln(I_0/I) = \sigma_\lambda C \ell, \quad (14)$$

where ℓ is the thickness of the gas absorption cell in cm, I_0 is the intensity of the light at $\ell = 0$, I is the intensity of light at thickness ℓ , C is the concentration of the gas in molecules cm^{-3} , and σ_λ is the cross section in the units $\text{cm}^2 \text{ molecule}^{-1}$ (the symbol conventionally used by most atmospheric chemist). Conversion of equation 14 to log to the base 10 yields

$$\log_{10}(I_0/I) = \sigma'_\lambda C \ell, \quad (14a)$$

where $\sigma'_\lambda = \sigma_\lambda/2.303$. Since A_λ , the absorbance (which is measured directly on most double beam uv-visible absorption spectrophotometers) is equal to $\log_{10}(I_0/I)$, equation 14a becomes

$$A_\lambda = \sigma'_\lambda C \ell. \quad (15)$$

For a 10 nm wavelength interval, equation 15 becomes

$$A_{\Delta\lambda} = \sigma'_{\Delta\lambda} C \ell. \quad (16)$$

The absorbance $A_{\Delta\lambda}$ can be obtained from the ultraviolet-visible absorption spectrum of the test chemical in the gas phase as described by Hendry [Mill et al. (1982)] and improved by Pitts et al. (1981). These data will be used in equation 16 to calculate the cross section for 10 nm wavelength intervals where

the test chemical absorbs light. The method outlined in this Test Guideline is a typical standard procedure for determining the cross section of chemicals in the gas phase.

The cross section of a test chemical in solution can be determined by the methods outlined in Test Guideline CG-1050.

c. Applicability and Specificity

This test method is applicable to all chemicals which have uv-visible absorptions in the range 290-800 nm. Solar radiation reaching the earth's surface has a sharp cutoff at a wavelength of approximately 290 nm due to the absorption by ozone [Leighton (1961), Peterson (1976), Zepp and Cline (1977), Demerjian (1980)]. The long wavelength limit is set by thermochemistry since light of wavelength greater than 800 nm is not of sufficient energy to break chemical bonds of ground state molecules [Calvert and Pitts (1966), Benson (1976)]. Photolysis does not occur unless there is absorption of radiant energy. This is a direct consequence of the Grottus-Draper law, the first law of photochemistry. If a chemical in the gas phase only absorbs light at wavelengths below 290 nm it will not undergo direct photolysis in sunlight. A few examples of chemicals that only absorb light below 290 nm and need not be tested in this Test Guideline are alkanes, alkenes, alkynes, dienes, and fluoroalkanes.

This Test Guideline is only applicable to pure chemicals and not to the technical grade. Overestimates of cross sections usually occur when technical grade substances are tested because the impurities frequently absorb in the same spectral region as the pure chemical.

This first-tier screening test can be employed to estimate $(k_pE)_{\max}$. and $(t_{1/2})_{\min}$. as a function of latitude and season of the year in the northern hemisphere under clear sky conditions. These data are in a form suitable for mathematical modeling for environmental fate of a test chemical.

If the data obtained from this Test Guideline indicate that gas phase photolysis is an important process relative to other gas phase transformation processes such as oxidation with OH and O_3 , then it is recommended that an upper tier photolysis test be carried out to determine the quantum yield and thus more precise environmentally relevant rate constants and half-lives.

B. Rationale for the Selection of Test Conditions

1. Ultraviolet-Visible Spectrophotometer

The measurement of absorption spectra in the ultraviolet and visible regions is carried out routinely in many commercial and research laboratories throughout the U.S.. Many spectrophotometers are commercially available to carry out these measurements but double beam spectrophotometers are highly recommended because they are easy to operate and have excellent precision and accuracy. These instruments can scan the wavelength region 270 - 800 nm with great precision and accuracy, the wavelength regions of importance in this Test Guideline, and can measure absorbance directly with good sensitivity. It is important that the spectrophotometer be able to attain a 90% separation of two

monochromatic spectral features approximately 4 nm apart, peak to peak (i.e., the resolution should be at least 4 nm). This resolution is necessary because gas phase spectra can exhibit complex fine structure due to vibrational-rotational interactions which are superimposed over the electronic transition. It is important to be able to measure weak absorption, since even weak absorption extended over the wavelength interval 290 - 350 nm can contribute significantly to the rate of absorption of sunlight and therefore can significantly effect the rate of photolysis. Consequently, the recommended double beam spectrophotometer should have an absorbance sensitivity of approximately 0.001 at a signal/noise ratio of unity.

2. Vapor and Liquid Cells

The recommended gas absorption cell is designed to measure the absorption spectrum of a gas in the region 270 - 800 nm and can be easily constructed in the laboratory. The pathlength of the cell should be at least 10 cm to make it easier to measure weak absorptions.

A matched reference cell is useful but not essential. However, the sample and reference cells should be very similar. Small spectral differences between the sample and reference cells can be compensated for by running a blank with both these cells in the spectrophotometer.

The gas absorption cell is designed with a Teflon stopcock (or a Pyrex "o" ring stopcock) and a Viton "o"-ring so that it can be easily attached and evacuated on a vacuum line and to introduce and to seal the gaseous test chemical in the absorption cell. Stopcocks and "o"-rings of this type do not require grease to make an effective seal. Thus, the potential adsorption of a gaseous organic test chemical by grease is eliminated.

Ten cm liquid absorption cells are readily available commercially.

3. Vacuum Gas-Handling System

The vacuum system recommended in this Test Guideline is commonly used in many commercial and research laboratories, is easy to construct, and simple to operate. The system should be constructed with Pyrex glass since the use of other materials, such as various metals, can lead to undesirable wall adsorption problems. The gas-handling system is designed to attain a vacuum of less than 10^{-5} torr (1.3×10^{-6} kPa). Teflon stopcocks (or Pyrex "o" ring stopcocks) and Viton "o"-rings are highly recommended since they will maintain a good vacuum without the use of stopcock grease and will eliminate adsorption of the gaseous organic test chemical by the grease.

Three pressure gauges have been recommended for use with this equipment. The thermocouple gauges are desirable to monitor the pressure in the range 10^{-3} to 1 torr (1.3×10^{-4} kPa to 0.13 kPa) while the ionization gauge is used to measure high

vacuum; i.e., pressures less than 10^{-3} torr (1.3×10^{-4} kPa). These two gauges are used to verify that the vacuum system is operating satisfactorily and contains no leaks. The third pressure gauge is needed to monitor the pressure of the test chemical and diluent in the range 0.01 to 760 torr (0.0013 to 101.3 kPa) and the recommended gauge is a capacitance manometer since it measures precise pressures in the recommended range and is easy to operate. The capacitance manometer, with its small exposed metal surface, compared to Boudin spiral absolute pressure gauges such as those made by Wallace and Tiernan and Texas Instruments, minimizes adsorption of the test chemical.

4. Preparation of Samples

The recommended procedure for the preparation of the gas phase test chemical sample is commonly used in many commercial and research laboratories and is easy to carry out. The concentration of the test chemical, based on the pressure registered on the capacitance manometer, can be obtained from the ideal gas law

$$PV = nRT \quad , \quad (17)$$

where P is the pressure in torr, V is the volume in cm^3 , n is the number of moles, R is the gas constant and is equal to $6.236 \times 10^4 \text{ cm}^3 \text{ torr } ^\circ\text{K}^{-1} \text{ g-mole}^{-1}$, and T is the room temperature in degrees Kelvin. The concentration in moles cm^{-3} is

$$C = \frac{n}{V} = \frac{P}{RT} \quad , \quad (18)$$

and the concentration in molecules cm^{-3} is

$$C = \frac{PN}{RT} \quad , \quad (19)$$

where N is 6.023×10^{23} molecules g-mole^{-1} and is Avogadro's number. Thus,

$$C \text{ (molecules cm}^{-3}\text{)} = 9.658 \times 10^{18} \frac{P(\text{torr})}{T(^{\circ}\text{K})} \quad . \quad (20)$$

For gas phase spectra with absorbance < 0.001 in a 10 cm cell, solution phase spectra are recommended to determine σ'_{λ} . While not capable of yielding an accurate value for σ'_{λ} , solution phase results are reproducible and yield an order-of-magnitude approximation [Pitts et al. (1981)].

Solution phase samples can be prepared in the standard manner in the recommended spectroscopic grade solvents chloroform, n-hexane, acetonitrile, and cyclohexane. These spectroscopic solvents are recommended because they are transparent in the wavelength region > 290 nm and many organic chemicals are sufficiently soluble in them. Solvents such as methanol or ethanol should be avoided because of their greater ability to perturb absorptions. Solvent effects can exist, however, and can affect the values of k_{PE} .

5. Determination of the Cell Pathlength

The method for determining the cell pathlength of a gas or liquid absorption cell is left to the discretion of the tester. However, the method listed in Test Guideline CG-1050, using one of the reference compounds, is highly recommended since it is a procedure commonly used by many commercial and research laboratories.

6. Deviation from the Beer-Lambert Law

At high pressures, gases may show deviation from the Beer-Lambert law. Thus, the pressure of the test chemical should be in the range 1 to 5 torr to minimize the deviation. If deviation from the Beer-Lambert law is observed, it is necessary to report the pressure at which the deviation was overcome and the experimental data.

If σ_{λ}' is determined from solution spectra, the concentration of the chemical should be in the range where the Beer-Lambert law is obeyed. If deviation from this law is observed, it is necessary to report the concentration at which the deviation is overcome and the experimental data.

7. Determination of Wavelength-AveragedSample Absorbances

There are at least three nondestructive methods recommended for determining an average absorbance over a specific wavelength region: estimation, square counting, and planimetry. The technique of cutting and weighing the spectrum is not recommended since it is destructive and liable to significant errors due to differing density of different portions of the paper on which the spectrum is obtained. For many spectra, estimating an average absorbance over a small wavelength interval is sufficient to yield accurate results. This is adequate for measuring absorbance of test chemicals in solution. However, this method is not the choice for gas phase spectra which may exhibit rapidly increasing and decreasing absorptions and complex fine structure. Thus, the other two methods of square counting or planimetry should be used. However, the method using a compensating polar planimeter is the recommended procedure since it is the most accurate method of determining the average absorbance for complicated spectra with fine structure.

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